University of Khartoum
The Graduate College
Medical and Health Studies Board

Analysis of some Pharmaceuticals utilizing Sequential Injection and Differential Electrolytic Potentiometry

By
Atta Elmanan Eldaw Ibrahim
B. Pharm. (U of K) 1986

A thesis submitted in fulfillment for the requirements of master in Pharmaceutical Chemistry

Supervisor
Professor Kamal Eldin Eltayeb Ibrahim
PhD
Professor of Pharmaceutical Chemistry
U of K

External supervisor
Professor Abdalla Abu-Alkibash
Professor of Chemistry
KFUPM

2009

المستخلص

الأهداف

من أهداف هذه الدراسة عمل طرق شاملة لأكسدة أدوية كلورفينارمين مليات و فيرابااميـل كلوريد الهايدروجين بواسطة بيرمنجنات البوتاسيوم في وجود حمض الكبريتيك باسـتخدام خاصـية الحقن المتعاقب الطيفي و بروميثازين كلوريد الهايدروجين بواسطة السيريم الرباعي في وجود حمض الكبريتيك بنظام فرق الجهد الكهربائي التفاضلي و كذلك انشاء و تركيب أنابيب متفرعـة ملائمـة و عمل برامج مناسبة للتحكم بهذه الطرق الجديدة إضافة إلى البحث عن ظروف التحليل المثلى و تطبيق المصفوفات الكيميائية للوصول لأفضل الظروف لطريقة الحقن المتعاقب الطيفي فقط و تقييم مـدى دقة و مصداقية و تكرار و تكلفة هذه الطرق و أخيرا مقارنتها مع دستور الأدوية البريطاني.

في هذه الأطروحة تم عمل نظامين، الأول هو استخدام تقنية التحليل بالحقن المتعاقب لأدوية كلورفينارمين مليات و فيرابااميل كلوريد الهايدروجين في المستحضرات الدوائيـة، و الآخـر هـو استخدام المعايرة بنظام فرق الجهد الكهربائي التفاضلي لعقار بروميثازين كلوريد الهايدروجين فـي المستحضرات الدوائية.

منهجية البحث

تعتبر طرق التحليل بالحقن المتعاقب طرق جديدة، سريعة، إقتصادية و ذاتيـة. تقـوم هـذه الطرق الجديدة باستخدام خاصية الحقن المتعاقب الطيفي و المصفوفات الكيميائية. تعتمد طريقتا تحليل كلورفينارمين مليات و فيرابااميل كلوريد الهايدروجين على أكسدتهما بواسطة بيرمنجنات البوتاسـيوم في وجود حمض الكبريتيك و رصد مقدار النقصان الناتج في إمتصاص البيرمنجنـات عنـد طـول موجي 546 نانومتر ، كما تم تطبيق المصفوفات الكيميائية للوصول لأفضل الظروف و هي طـرق إستجابة السطح و تأثير العوامل كأجزاء من تحليل التغير. كذلك تم إختيار تركيز الحمض و تركيـز البيرمنجنات و وقت التحليل كعوامل مؤثرة في كفاءة التحليل، و تم إستخدام العامـل 2^3 فـي هـذا التحليل ووجد من تقدير تأثير العوامل و التأثيرات المتداخلة في توافق مع إستجابة السطح و ان تأثير تركيز البيرمنجنات ووقت التحليل اكبر من تأثير تركيز الحمض.

تعتبر طريقة التحليل بواسطة فرق الجهد الكهربائي التفاضلي من الطرق الجديدة حيـث أن أغلب الباحثين يستخدم طرق التحليل التقليدية بواسطة فرق الجهد الكهربائي بدون تيار و باسـتخدام قطب قياسي و آخر عملي بينما المعايرة في تفاعلات الأكسدة يمكننا إستخدام قطبين متطـابقين مـن البلاتين لقياس فرق الجهد الكهربائي التفاضلي في وجود تياركهربائي قليل. و قد تم إسـتخدام هـذه الطريقة لتحليل عقار بروميثازين كلوريد الهايدروجين في المستحضرات الدوائية بأكسدته بواسـطة السيريم الرباعي في وجود حمض الكبريتيك و قياس فرق الجهد و الحجم الناتجين من هذه المعايرة.

النتائج

لقد وجد أن أفضل ظروف التحليل الكمي لكلورفينارمين هي 35 ميكروليتر مـن 1×10^{-3} مول لكل لتر من الحمض و 30 ميكروليتر من 1×10^{-3} مول لكل لتر من البيرمنجنات و 4 دقائق وقت تحليل و 30 ميكروليتر من كلورفينارمين و معدل انسياب 25 ميكروليتر لكل ثانيـة لتعطـي معادلة تعيير ($R = 0.0016C + 0.0064$) و معامل ارتباط (0.9998) و مدى خطي من 20 الى 150 جزء من المليون.

و أن أفضل ظروف التحليل الكمي لفيراباميل هي 35 ميكروليتر من 1×10^{-3} مول لكـل لتر من الحمض و 30 ميكروليتر من 2×10^{-3} مول لكل لتر من البيرمنجنات و دقيقتان وقت تحليل و 30 ميكروليتر من فيراباميل و معدل انسياب 25 ميكروليتر لكل ثانيـة لتعطـي معادلـة تعييـر ($R = 0.0018C + 0.005$) و معامل ارتباط (0.9978) و مدى خطي من 50 الى 200 جزء من المليون.

تبنى طريقة التحليل الكمي لبروميثازين بنظام فرق الجهد الكهربائي التفاضلي على أكـسدته بواسطة السيريم الرباعي في وجود حمض الكبريتيك و قياس فرق الجهد و الحجم الناتجين من هـذه المعايرة و من ثم رسم منحنيات المعايرة المختلفة باستخدام فرق الجهد الناتج و حجم السيريم الرباعي اللازم لاتمام المعايرة ثم دراسة النتائج و عمل الحسابات اللازمة، و لقد وجد أن جزءاً واحداً مـن البروميثازين قد تفاعل مع جزءاً واحداً من السيريم الرباعي. ووجد أن أفضل ظروف التحليل على نحو ما يلي: تركيز الالكترولايت المعاضد و هو حمض الكبريتيك يتراوح بين 1×10^{-3} - 5×10^{-3} مول لكل لتر، تركيز السيريم الرباعي يتراوح بين 5×10^{-5} - 1×10^{-3} مول لكـل لتـر، و تيـار متردد 5 مايكروأمبير و معدل انسياب 20 ميكروليتر لكل ثانية.

الخلاصة

لقد وجد أن الطرق الجديدة ذات دقة و مصداقية عند مقارنتهـا بنتـائج دسـتور الأدويـة البريطاني و كذلك فيما يتعلق بسرعتها و معالجتها للعينات و توفيرها للكواشف و محافظتهـا علـى البيئة و أيضا من ناحية الذاتية و الانتقائية اضافةً الى الدقة و المصداقية.

تعتبر طريقة التحليل بواسطة فرق الجهد الكهربائي التفاضلي الجديدة عالية الحـساسية مـع درجات كشف منخفضة الحدود، و لكنها قد تتأثر كثيراً بتغير تركيز الالكترولايت المعاضـد لهـذا يجب ضبطه بعناية تامة لضمان التكرار. و لقد وجد أن النتائج المتحصل عليها من هـذه الطريقـة يمكن مقارنتها بتلك المنصوص عليها في دستور الأدوية البريطاني مع ميزة كونها اقتصادية، دقيقة و ذات مصداقية و شبه ذاتية.

Abstract

Objectives

The objectives of this study is to perform comprehensive procedures for the oxidation of chlorpheniramine maleate and verapamil hydrochloride by permanganate in sulphuric acid media utilizing sequential injection analysis (SIA) and promethazine hydrochloride by cerium IV in acid medium using differential electrolytic potentiometry (DEP). To construct SIA and DEP manifolds suitable for these methods of assay. To establish appropriate programs to control the newly adopted methods. To search for the optimum conditions for these assay methods, to conduct a chemometric optimization for the SIA methods, to evaluate the accuracy, precision, repeatability, robustness and cost of the newly adopted method, and finally to validate the newly adopted methods by comparison with the BP standard methods.

In this thesis two systems were studied, the first was sequential injection analysis (SIA) technique for the assay of chlorpheniramine maleate and verapamil hydrochloride in pharmaceuticals formulations, and the second was DEP (differential electrolytic potentiometry) for the assay of promethazine hydrochloride in pharmaceutical formulations.

Methodology

SIA methods were novel, robust, cost-effective and fully automated. The newly adopted methods utilized sequential injection analysis with spectrophotometry and chemometric approaches. The methods for the assay of chlorpheniramine and verapamil were based on their oxidation by permanganate in sulfuric acid medium and their consequent spectrophotometric measurement of the absorbance at wavelength 546 nm. The methods were optimized chemometrically with surface response and estimation of effects factorial design ANOVA approaches. This method was applied for factors which presumably have potential effects on the efficiency of the methods, such as acid and permanganate concentrations and delay time. A 2^3 factorial design

chemometric method was applied, that from the estimation of effect factors, the main and interaction effect factors were found to be in agreement with the surface response. These findings indicate that the concentration of permanganate and delay time have a greater effect on response than acid concentration.

DEP method is considered new since researchers are still using the traditional potentiometry at zero current with standard and working electrodes. In DEP for redox titration two identical platinum electrodes at low current are used. The assay of promethazine hydrochloride was based on its oxidation by cerium (IV) in sulfuric acid medium and consequent measurement of the potential difference and recording the volume of cerium (IV) needed to complete the titration.

Results

In the chlorpheniramine maleate assay, the optimum operating conditions were found to be 35µl of 1×10^{-3} mol l^{-1} sulfuric acid, 30µl of 1×10^{-3} mol l^{-1} permanganate, 4 minutes delay time and 30µl of chlorpheniramine with a flow rate of 25µl s^{-1}. Beer' s law was obeyed in the concentration range of 20 – 150 ppm with regression calibration equation (R=0.0016C+0.0064) and correlation coefficient of 0.9998.

In the verapamil hydrochloride assay, the optimum operating conditions were found to be 35µl of 1×10^{-3} mol l^{-1} sulfuric acid, 30µl of 2×10^{-3} mol l^{-1} permanganate, 2 minutes delay time and 30µl of verapamil with a flow rate of 25µl s^{-1}. Beer' s law was obeyed in the concentration range of 50 – 200 ppm with regression calibration equation (R=0.0018C+0.005) and correlation coefficient 0.9978.

In DEP different titration curves of volume of cerium (IV) against potential were drawn. The results obtained were studied and the necessary calculations were made. It was found that one part of promethazine reacts with one part of cerium (IV). The optimum working conditions were as follows for supporting electrolyte concentration sulfuric acid, ranges from $1\times10^{-}3$mol l^{-1}-

5×10^{-3}mol l^{-1}, cerium (IV) concentration ranges from 5×10^{-5} mol l^{-1}-1×10^{-3} mol l^{-1}, DC current of 5µA and flow rate of 20µl s^{-1}.

Conclusion

The developed SIA methods were found to be accurate and reproducible when the results were statistically compared with the results obtained by the official standard methods with respect to sample handling, reagents saving, environmental hazardous, automation, precision and accuracy.

The developed DEP method was found to be highly sensitive with lower detection limits and seemed to be largely affected by the variation of supporting electrolyte concentrations which may affect repeatability and should be carefully adjusted. The results obtained from DEP method were comparable to those of the official BP methods, with the advantages of being economical, accurate, precise and partially automated.

Acknowledgements

I would like to express my sincere gratitude and thanks to my supervisor professor Kamal E. Ibrahim for his guidance, cooperation and assistance to accomplish this work with such great success.

My thanks and gratitude are also extended to my co-supervisor professor Abdalla Abu-Alkibash, Chemistry Department, KFUPM, Dhahran, K. S. A., for his continued encouragement, support and patience.

My thanks and gratitude are also extended to the chairman Dr. Abdalla Al-Hamdan and the staff of Chemistry Department, KFUPM, Dhahran, K. S. A., for allowing me to do the practical work at their laboratories and to use the facilities available.

I would like also to convey my thanks to professor Salah M. Sultan and Dr. Abubakr M. Idris for their great help and support.

.

الخلاصة

في هذه الأطروحة تم عمل نظامين، الأول هو استخدام تقنية التحليل بالحقن المتعاقب لأدوية كلورفينارمين مليات و فيرابااميل كلوريد الهايدروجين في المستحضرات الدوائية، و الآخر هو استخدام المعايرة بنظام فرق الجهد الكهربائي التفاضلي لعقار بروميثازين كلوريد الهايدروجين في المستحضرات الدوائية.

تعتبر طرق التحليل بالحقن المتعاقب طرق جديدة، سريعة، إقتصادية و ذاتية. تقوم هذه الطرق الجديدة باستخدام خاصية الحقن المتعاقب الطيفي و المصفوفات الكيميائية. تعتمد طريقتا تحليل كلورفينارمين مليات و فيرابااميل كلوريد الهايدروجين على أكسدتهما بواسطة بيرمنجنات البوتاسيوم في وجود حمض الكبريتيك و رصد مقدار النقصان الناتج في إمتصاص البيرمنجنات عند طول موجي 546 نانومتر ، كما تم تطبيق المصفوفات الكيميائية للوصول لأفضل الظروف و هي طرق إستجابة السطح و تأثير العوامل كأجزاء من تحليل التغير. كذلك تم إختيار تركيز الحمض و تركيز البيرمنجنات و وقت التحليل كعوامل مؤثرة في كفاءة التحليل، و تم إستخدام العامل 2^3 في هذا التحليل ووجد من تقدير تأثير العوامل و التأثيرات المتداخلة في توافق مع إستجابة السطح و ان تأثير تركيز البيرمنجنات ووقت التحليل اكبر من تأثير تركيز الحمض.

لقد وجد أن أفضل ظروف التحليل الكمي لكلورفينارمين هي 35 ميكروليتر من 1×10^{-3} مول لكل لتر من الحمض و 30 ميكروليتر من 1×10^{-3} مول لكل لتر من البيرمنجنات و 4 دقائق وقت تحليل و 30 ميكروليتر من كلورفينارمين و معدل انسياب 25 ميكروليتر لكل ثانية لتعطي معادلة تعيير ($R = 0.0016C + 0.0064$) و معامل ارتباط (0.9998) و مدى خطي من 20 الى 150 جزء من المليون.

و أن أفضل ظروف التحليل الكمي لفيرابااميل هي 35 ميكروليتر من 1×10^{-3} مول لكل لتر من الحمض و 30 ميكروليتر من 2×10^{-3} مول لكل لتر من البيرمنجنات و دقيقتان وقت تحليل و 30 ميكروليتر من فيرابااميل و معدل انسياب 25 ميكروليتر لكل ثانية لتعطي معادلة تعيير $R = (0.005 + 0.0018C)$ و معامل ارتباط (0.9978) و مدى خطي من 50 الى 200 جزء من المليون.

لقد وجد أن الطرق الجديدة ذات دقة و مصداقية عند مقارنتها بنتائج دستور الأدوية البريطاني و كذلك فيما يتعلق بسرعتها و معالجتها للعينات و توفيرها للكواشف و محافظتها على البيئة و أيضا من ناحية الذاتية و الانتقائية اضافةً الى الدقة و المصداقية.

تعتبر طريقة التحليل بواسطة فرق الجهد الكهربائي التفاضلي من الطرق الجديدة حيث أن أغلب الباحثين يستخدم طرق التحليل التقليدية بواسطة فرق الجهد الكهربائي بدون تيار و باستخدام قطب قياسي و آخر عملي بينما المعايرة في تفاعلات الأكسدة يمكننا إستخدام قطبين متطابقين من البلاتين لقياس فرق الجهد الكهربائي التفاضلي في وجود تياركهربائي قليل.

تبنى طريقة التحليل الكمي لبروميثازين على أكسدته بواسطة السيريم الربـاعي فـي وجـود حمض الكبريتيك و قياس فرق الجهد و الحجم الناتجين من هذه المعايرة و من ثـم رسـم منحنيـات المعايرة المختلفة باستخدام فرق الجهد الناتج و حجم السيريم الرباعي اللازم لاتمام المعايرة ثم دراسـة النتائج و عمل الحسابات اللازمة، و لقد وجد أن جزءاً واحداً من البروميثازين قد تفاعل مع جـزءاً واحداً من السيريم الرباعي. ووجد أن أفضل ظروف التحليل على نحو ما يلي: تركيز الالكتروليـت المعاضد و هو حمض الكبريتيك يتراوح بين 1×10^{-3} - 5×10^{-3} مول لكل لتـر، تركيـز الـسيريم الرباعي يتراوح بين 5×10^{-5} - 1×10^{-3} مول لكل لتر، و تيار متـردد 5 مـايكروأمبير و معـدل انسياب 20 ميكروليتر لكل ثانية.

تعتبر طريقة التحليل بواسطة فرق الجهد الكهربائي التفاضلي الجديدة عالية الحـساسية مـع درجات كشف منخفضة الحدود، و لكنها قد تتأثر كثيراً بتغير تركيز الالكتروليت المعاضد لهذا يجب ضبطه بعناية تامة لضمان التكرار. و لقد وجد أن النتائج المتحصل عليها من هـذه الطريقـة يمكـن مقارنتها بتلك المنصوص عليها في دستور الأدوية البريطاني مع ميزة كونها اقتصادية، دقيقـة و ذات مصداقية و شبه ذاتية.

Abstract

In this thesis two systems were studied, the first was sequential injection analysis (SIA) technique for the assay of chlorpheniramine maleate and verapamil hydrochloride in pharmaceuticals formulations, and the second was DEP (differential electrolytic potentiometry) for the assay of promethazine hydrochloride in pharmaceutical formulations.

SIA methods were novel, robust, cost-effective and fully automated. The newly adopted methods utilized sequential injection analysis with spectrophotometry and chemometric approaches. The methods for the assay of chlorpheniramine and verapamil were based on their oxidation by permanganate in sulfuric acid medium and their consequent spectrophotometric measurement of the absorbance at wavelength 546 nm. The methods were optimized chemometrically with surface response and estimation of effects factorial design ANOVA approaches. This method was applied for factors which presumably have potential effects on the efficiency of the methods, such as acid and permanganate concentrations and delay time. A 2^3 factorial design chemometric method was applied, that from the estimation of effect factors, the main and interaction effect factors were found to be in agreement with the surface response. These findings indicate that the concentration of permanganate and delay time have a greater effect on response than acid concentration.

In the chlorpheniramine maleate assay, the optimum operating conditions were found to be 35µl of 1×10^{-3} mol l^{-1} sulfuric acid, 30µl of 1×10^{-3} mol l^{-1} permanganate, 4 minutes delay time and 30µl of chlorpheniramine with a flow rate of 25µl s^{-1}. Beer' s law was obeyed in the concentration range of 20 – 150 ppm with regression calibration equation (R=0.0016C+0.0064) and correlation coefficient of 0.9998.

In the verapamil hydrochloride assay, the optimum operating conditions were found to be 35µl of 1×10^{-3} mol l^{-1} sulfuric acid, 30µl of 2×10^{-3} mol l^{-1} permanganate, 2 minutes delay time and 30µl of verapamil with a flow rate of 25µl s^{-1}. Beer' s law was obeyed in the concentration range of 50 – 200 ppm with

regression calibration equation (R=0.0018C+0.005) and correlation coefficient 0.9978.

The developed SIA methods were found to be accurate and reproducible when the results were statistically compared with the results obtained by the official standard methods with respect to sample handling, reagents saving, environmental hazardous, automation, precision and accuracy.

DEP method is considered new since researchers are still using the traditional potentiometry at zero current with standard and working electrodes. In DEP for redox titration two identical platinum electrodes at low current are used. The assay of promethazine hydrochloride was based on its oxidation by cerium (IV) in sulfuric acid medium and consequent measurement of the potential difference and recording the volume of cerium (IV) needed to complete the titration. Different titration curves of volume of cerium (IV) against potential were drawn. The results obtained were studied and the necessary calculations were made. It was found that one part of promethazine reacts with one part of cerium (IV). The optimum working conditions were as follows for supporting electrolyte concentration sulfuric acid, ranges from $1\times10^{-}3$mol l^{-1}-5×10^{-3}mol l^{-1}, cerium (IV) concentration ranges from 5×10^{-5} mol l^{-1}-1×10^{-3} mol l^{-1}, DC current of 5μA and flow rate of 20μl s^{-1}.

The developed DEP method was found to be highly sensitive with lower detection limits and seemed to be largely affected by the variation of supporting electrolyte concentrations which may affect repeatability and should be carefully adjusted. The results obtained from DEP method were comparable to those of the official BP methods, with the advantages of being economical, accurate, precise and partially automated.

CONTENTS

Chapter two: Materials and methods

Chapter three: Results and discussion

List of figures

List of tables

CHAPTER ONE

INTRODUCTION

1.1 Principles of Flow injection (FI) techniques

All flow injection techniques are based on the injection of a liquid sample into a moving, non-segmented carrier stream of a suitable liquid. The injected sample form a zone, which is then transported to a detector that records a desired physical parameter as it is continuously changing due to the passage of the sample material through a flow cell.

Since it is introduced in the year 1974, FI technique has grown dramatically and undergone a lot of modifications and developments, and it is classified into three generations; the first generation is flow injection analysis (FIA), the second generation is sequential injection analysis (SIA), and the third one is bead injection analysis-lab-on-valve (BIA-LOV). The following sections briefly describe the main principles, features and the developments of the technique as well as its advantages and limitations.

1.1.1 Flow injection analysis (FIA)

Ruzicka and Hansen in Denmark as well as, Steward in the US created the flow injection analysis (FIA) technique for the automation of chemical procedures [1, 2].The introduction of this technique revolutionized the concept of automation in chemical analysis by allowing instrumental measurements to be carried in the absence of physical and chemical equilibrium [3-7]. FIA became popular since it automates routine procedures with reduction in sample consumption and providing high sampling rates, in addition to its use in other areas of chemical analysis, such as the study of kinetic and mechanistic aspects of chemical reactions and the development of new chemical, enzymatic and immunological procedures.

The FIA method combines several analytical functions for a method performed in a flowing stream and under computer control, in a time of 15-60 seconds with the volume of a sample in microliters with a simple hardware and high precision of about 0.5% (R. S. D.) with relatively little waste and broad concentration range form ppb to %(w/v). A FIA method can be divided into three stages. Figure 1.1 shows the components of the FIA manifold. The first

stage and the second are the heart of FIA, while the third one is based on conventional technology. All stages are performed in sequence in a flowing carrier stream pumped through a narrow bore tubing of 0.5 mm I.D. The first step is the injection of a measured sample into a flowing carrier stream (injected sample volumes are 50 to 200 µL, while a typical sampling frequency is 60 injections/ hour). Samples are injected by means of a two-position valve with a fixed internal volume (loop). The valve is furnished with a bypass that allows the loop to be filled while the carrier stream is passing through the valve. The second stage is a sample processing in which the analyte is transformed into species that can be detected and have its concentration manipulated into a range that is compatible with the detector. The third one is the detection where the analyte or one of its derivatives produces a response that can be reliably quantified.

FIA has also been used as a "front end" or solution handling system, for an entire range of spectroscopic and electrochemical instruments, since it offers sample pretreatment, matrix removal and automated recalibration of different detectors. FIA can be easily configured with almost all spectroscopic techniques including UV/VIS spectrophotometry, fluorescence, chemiluminescence, atomic absorption spectrophotometry, atomic emission spectrophotometry and nephelometry. In addition, electrochemical measurements like potentiometry, voltammetry, polarography and conductimetry. FIA as an analytical tool rapidly and automatically performs methods with less sample and reagent consumption [4].

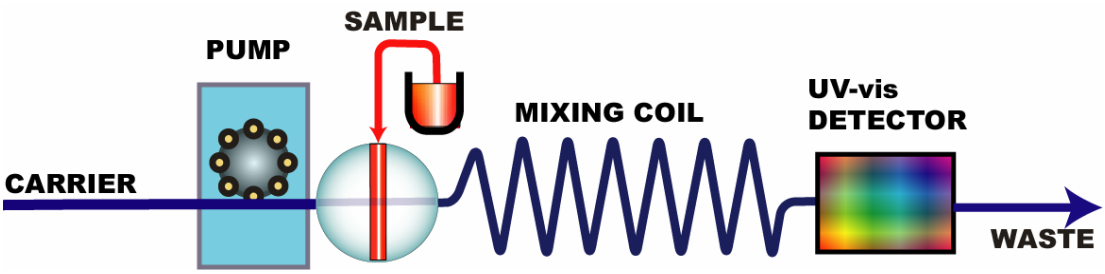


Figure 1.1: single stream FIA manifold

1.1.2 Sequential injection analysis (SIA) [7-15]

Following the general applications of computers in the routine laboratory work, it was possible to cover up the deficiencies of the FIA technique in autonomy, given that automatic control of the different electric components in the mountings was possible. In that way, a second generation of flow analysis was proposed by Ruzicka and Marshal in 1990, designated as sequential injection analysis (SIA) [8]. As with the FIA, this is a non-segmented continuous flow technique based on the same principles of controlled dispersion and reproducible manipulation of the FIA concept, but whose mode of functioning is based on the concept of programmable flow. A basic SIA system (figure 1.2) consists of a bi-directional propulsion device, a holding coil, a multiposition selection valve, a detector, tubing adequate for unifying all different components of the system and a microcomputer.

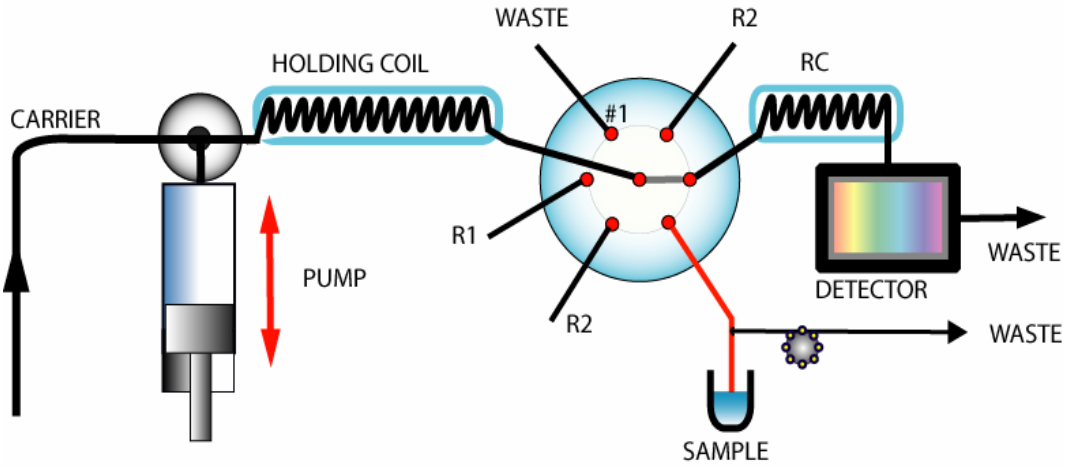


Figure 1.2: Basic SIA manifold; RC is reaction coil; R1 and R2 are reagents

This component makes the synchronized control of the propulsion device and the multiposition selection valve possible in such a way to define the volume and direction of stream of the different solutions. In a typical analytical cycle, precise volumes of sample and reagents are sequentially aspirated through the multiposition selection valve to the holding coil. By commutation of the selection valve and by inversing the direction of the stream, a zone is created

(composed of the overlapping sample and reagents zones) for which the radial and axial dispersion processes also contribute. When the overlapping zone is directed to the detector, a transient signal is obtained of a magnitude proportional to the concentration of the species under study.

The introduction of SIA marked a new era in the development of flow analysis. Compared with FIA, SIA economically uses fewer reagents and reduces waste generation, because a permanent flow of carrier solution ceases to be necessary, so that the reagents and samples are used in a more efficient way, since only volumes necessary are processed. SIA can undertake analytical measurements on a large scale because of its high sampling rate (although the rates are less compared with FIA). SIA can easily be applied in the kinetic studies of chemical reactions, since the factors that affect the precision of the volumes and reaction times are rigorously controlled, [16, 17]. The experimental parameters can be altered and controlled easily by computer. Knowing the flow rate profiles and the selection of both time/flow rate using just one propulsion unit, makes these systems simple, robust and stable in the long term. The simplicity in configuration does not require significant reconfigurations, so any alteration in experimental conditions such as injection volume, reaction type, sample dilution or sample/reagent relation can be achieved by changing the instruction introduced by the computer keyboard. The versatility shown by SIA makes it possible to automate various steps of analytical procedures, for example: sample dilution [18], automatic calibration [19], liquid-liquid extraction [20], gas-liquid diffusion [21], dialysis and on-line digestion.

In the course of 17 years, SIA has demonstrated itself to be a powerful and versatile instrument for the automation of different analytical procedures. This technique has been associated with a large variety of detectors including UV/VIS spectrophotometry, fluorescence, turbidimetry, chemiluminescence, atomic absorption spectrophotometry, mass spectrometry with radio-frequency plasma, atomic emission spectrophotometry, and electrochemical measurements like potentiometry, voltammetry, polarography and conductimetry. It has been applied in diverse areas of chemistry like, environmental, pharmaceutical, food

and beverages, biotechnology, radiochemical and metallurgic, kinetic studies of chemical reactions and chemometric studies.

1.1.3 Sequential injection analysis-lab-on-valve (SIA-LOV)

In order to overcome the drawbacks (handling of hazardous chemicals, in reagent-limited assays or waste production as a critical parameter) a new generation of flow injection analysis techniques namely sequential injection analysis-lab-on-valve (SI-LOV) was introduced. The LOV system potentially offers facilities of allowing any kind of chemical and physical process, including fluidic and microcarrier bead control, homogenous reaction, , liquid-solid extraction, and in-valve, real time optical detection of various reaction processes with optical fibers . SIA-LOV has already proved to be an attractive, effective front end to various detection devices with microminiaturized sample processing along with improved efficiency and ruggedness. Figure 1.3 shows a typical micro SIA-LOV system with a close-up of the multi-purpose flow cell configured for real-time measurement of absorbance [22, 23].

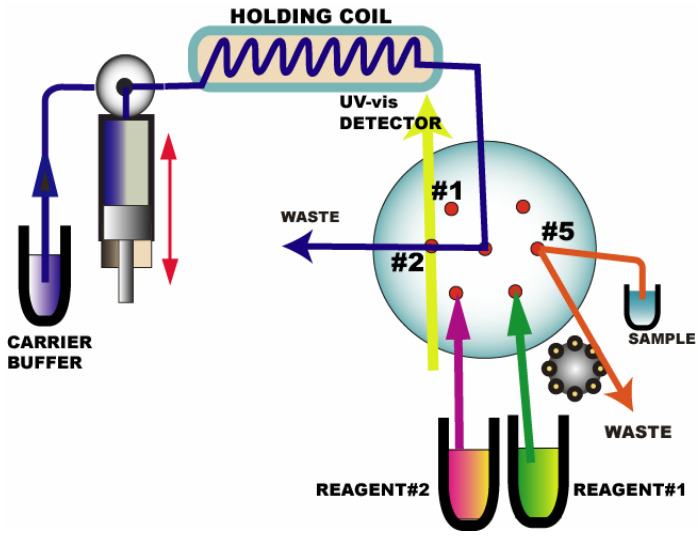


Figure 1.3: A typical micro SIA-Lab-On-Valve

1.1.4 Sequential injection chromatography (SIC)

A new generation of sequential injection analysis (SIA) called sequential injection chromatography (SIC) has already been introduced as a good alternative of high performance liquid chromatography (HPLC) for fast analysis of simple samples. The benefits of this flow method are automation, miniaturization and low sample and mobile phase consumption. The implementation of short monolithic chromatographic column into SIA opens new areas for on-line chromatographic separation of multi-compound sample in low-pressure flow system, with the advantage of programmable flow and possibility of sample manipulation. The potentials of SIC and its comparison with HPLC for the determination of pharmaceuticals was discussed, but recent trends focused on separation with SIC.

Sequential injection chromatography (SIC) has been successfully applied in the analysis of relatively simple multi-component samples, mainly in the field of pharmaceutical analysis. Sequential injection chromatography is built on classical SIA manifold (in our case FIAlab® 3000 or FIAlab® 3500 analyzers equipped with syringe pump). Chromatographic part is represented by short (usually 25 or 50mm of length) commercially available monolithic column (with or without monolithic pre column 5 or 10mm length) placed between multi-position valve and Z flow cell of detector (Figure 1.4) [24].

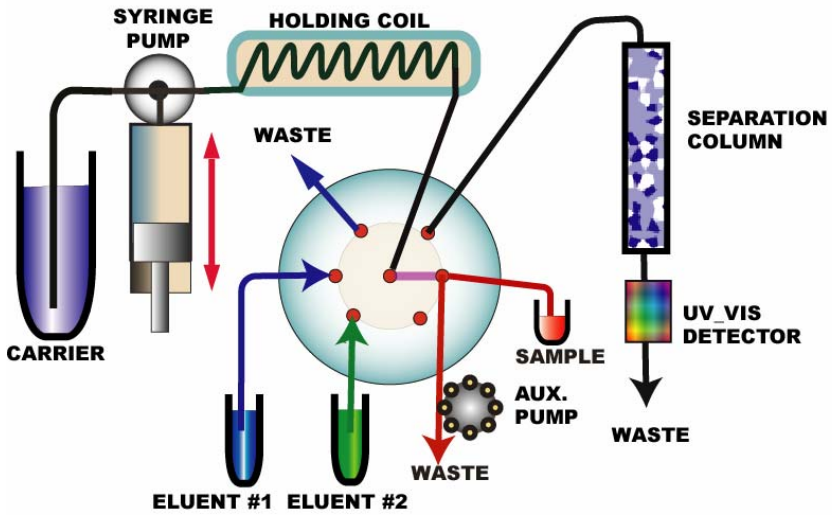


Figure 1.4: Sequential injection chromatography (SIC)

1.1.5 Bead injection analysis (BIA)

Bead injection analysis (BIA), as a third generation of FI techniques, first introduced by Ruzicka in 2000, has a programmable flow to handle precise volumes of suspended micro beads to perform such physical or chemical processes for analytes. Beads can be used as a removable solid surface in many analytical applications. It is very practical to use beads in a flow based system, where beads can easily be moved in and out of the system using a flowing stream of reagent(s). The use of beads for sample pretreatment, such as preconcentration, isolation, and separation, and for accommodation of chemical reactions has attracted increasing interest. The development and applications of the BI technique coupled with a lower cost first-generation FI technique is a good alternative to other expensive systems. In its simplest form, microspheres are injected into a conduit, where they can be trapped at a selected location, then the sample zone is injected and perfused through the beads where sample components react with functional groups on bead surfaces, and retained analytes are detected by spectroscopy. Analyte molecules may also be eluted for detection downstream. The unique features of bead injection are high precision of bead delivery, absence of carryover and fast-automated renewal of the reactive solid phase that is delivered in uniform composition throughout a series of measurements (figure 1.5) [25].

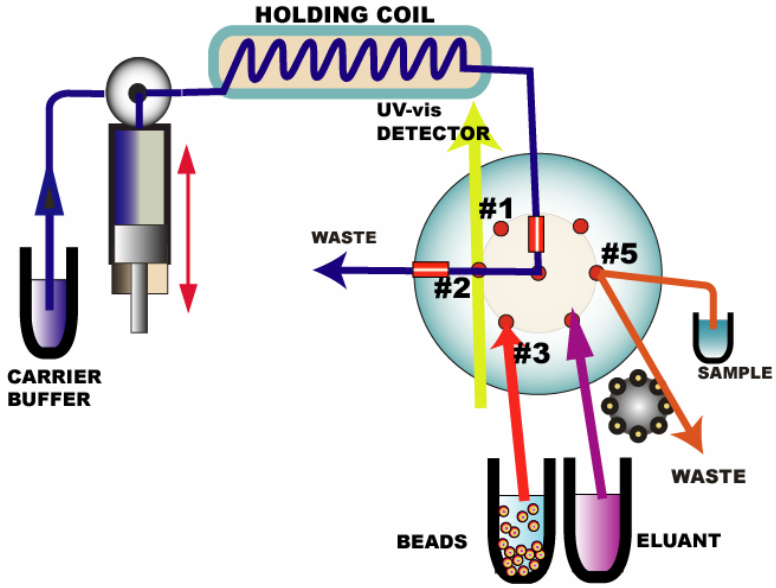


Figure 1.5: Bead injection analysis (BIA)

1.2 Dispersion in FI technique

Any injection analyzer requires for its successful operation to have the capability to bring about the sample and the reactants together and allow them to be mixed and react in a reproducible manner. In this respect flow conditions should be maintained constant for specific period of time, and as long as flow-rate is kept constant the sample may be injected into the system at any time, so what happens to one sample happens exactly to any other sample regardless to when the sample is injected into the carrier stream. To fulfill the above, strict control of the flow rate must be maintained throughout the entire analysis process. [5].

In FIA the sample is completely surrounded by reagents, while in SIA the sample and reagent zones are sequentially staked and moved along the instrument, the zones penetrate each other and become interdispersed. The penetration degree has been measured and defined to help understand zone dispersion. These include: D (dispersion); $S_{1/2}$ (volume required to reach dispersion of 2 at the peak maximum); P (degree of zone penetration). [26].

Gubeli et al [27] have observed that increasing the sample volume up to $S_{1/2}$ enhanced the sensitivity. However, beyond that volume ($S_{1/2}$) there was no improvement.

Marshal and van Staden [28] evaluated the precision and zone penetration to determine the effect of tubing diameter, reactor geometry and pump speed. Their conclusion showed that tubing with internal diameter of 0.8 or 1.5 mm gave improved precision without excessive decrease in zone penetration compared with that of 0.5mm. They found that knitted reactors were interfered to straight tubing with respect to axial dispersion, while the effect of pump speed was little as the flow rate changed due to its sinusoidal nature.

Clardera et al [29] studied the effect of ionic strength, flow rate and reactor and holding coils dimensions on the $S_{1/2}$ value and they found that the length and the inner diameter have some influence, but each system should be optimized separately.

1.3 Applications of SIA to pharmaceutical analysis

The FIA technique contributes largely to the development of automation in pharmaceutical analysis and its advantages are well documented. Pharmaceutical applications of FIA were summarized in a monograph [30] and updated elsewhere [31, 32]. The introduction of SIA has awakened the interest and the need for the automation in the pharmaceutical area. Two review articles dedicated to pharmaceutical analysis have been published [33,34], applying sequential analysis to a wide variety of matrices, solid (tablets, capsules), liquids (suspensions, solutions, emulsions), pastes (ointments, creams) and covering different active ingredients. Taking advantages of the economy of reagents and high sampling rates, most of these applications are used to determine the active ingredients for the quality control of pharmaceuticals. Other applications used its robustness and elevated efficiency to monitor the production of pharmaceutical compounds and for their dissolution assays.

1.3.1 Automated analysis of drug formulations

Determination of the active ingredients of pharmaceuticals is one of the main quality control tests, so the method used must be selective, precise and accurate, since additive substances are also present, (filers, binders, suspending agents and preservatives). SIA technique shows a great satisfaction for these requirements. The most recent review was published by Pimenta et al, [34] and it is summarized in table 1.1 for optical detection methods and table 1.2 for electrochemical detection methods.

1.3.1.1 Spectrophotometry

The majority of the determinations of active ingredient in pharmaceuticals belong to a group of spectrophotometric ones, and most of them are based on chromogenic reactions or on the specific characteristics of light absorption by analytes. The configuration of the SIA is normal, simple and requires the selection of the nature and volume to adapt to one or more chromogenic reagents and the detection with spectrophotometers. The chromogenic reactions for drugs

include among other procedures metal ion chelation reactions for the determination of organic compounds or metal ion components, redox reactions, ion pairing, charge transfer complex formation reactions.

1.3.1.2 Fluorescence and Chemiluminescence

In Fluorimetry, the procedures involve elevated selectivity, and it is more sensitive than that of the spectrophotometric ones by lowering the interference of the matrix and by the proportionality between the intensity of the radiation source and the analytical signal measured.

Chemiluminescent processes are classified into two groups, one in which the analyte itself is the emission specie through the direct action of strong oxidizing agents (mainly permanganate and CeIV). The other covers indirect methods through which the analyte interferes (as a catalizer, inhibitor, etc.) in the oxidation of the chemiluminescent precursors such as luminal, lucigenine, etc. The latter are called (classics). The current interests focus on liquid phase analytical chemiluminescence to find new chemiluminescent reactions directly on the analyte.

1.3.1.3 Electrochemistry

Electrochemical methods are favorable with flow analysis due to their sensitivity, controllable selectivity, precision and accuracy, simplicity with easy signal handling and automation. However the sensors are difficult to develop and the interaction between the sensors and the liquid tested lead to poor stability and reproducibility of the active electrode surface. These problems can be overcome by the rigorous selection of the experimental conditions. The sensors used with SIA gave robust, portable low-cost and high-throughput systems. SIA systems with voltammetric, potentiometric and amperometric detection are found in the literature (table 1.2).

A review of SI techniques applied to pharmaceutical analysis [34]

Table 1.1 Optical detection methods

Analyte	Matrix	Detection method	Linear range	R.S.D. %	Sample frequency h^{-1}	Ref.
Alendronic	Tablets	Spect. determ.*at (240 nm)	1.0-60.0 mg/l	3	60	[35]
acid		Fluori. det.¤ (λ_{ex}340;λ_{em}455nm)	0.13-10 mg/l	2	30	[35]
Ambroxol hydrochloride	Capsules Tablets	Spect. determ.*at (213 nm)	2-100 µg/ml	0.5-5.4	7	[36]
p-Aminobenz-	Sunscreens	Spect. determ.*at (500 nm)	0-25 µg/l	2.0- 6.0	13.8	[37]
oic acid	Lotain&sunmilk	Spect. determ.*at (434 nm)	0-20 µg/l	3.0-7.0	13	[38]
Amincaproic acid	Pharmaceutical formulations	Fluori. det.¤(λex350;λem450nm)	$4x10^{-7}$-$6x10^{-5}$mol/l	<2.0	40	[39]
Amoxycillin	Tablets	Spect. determ.*at (250 nm)	10-120mg/l	6.0-9.0	20	[40]
	Tablets	Spect. determ.*at (210-340 nm)	0-60mg/l	1.3	25	[41]
Benzocaine	Injections	Oxidation of drug bypermang- nate in acidic medium, chemiluminiscence (≥390nm)	0.5-25 mg/l	<3.8	120	[42]

Benzo-phenone-4	Sunscreens	Spect. determ.*at (286 nm)	10-120 µg/l	1.0-12.0	20	[43]
Bismuth	Tablets & caps	Spect. determ.*at (548 nm)	0.0-75 mg/l	1.1	72	[44]
Bopindolol	Tablets	Spect. determ.*at (264 nm)	1-10 µg/ml	<1	40	[45]
Boric acid	Eye solutions	Complexation between D-sorbitol and boric acid, then acid-base reaction Spect. determ.*at (520 nm)	0-12mg/l	<0.6	30	[46]
Boric acid	Eye solutions mineral waters	Fluori.det.¤ (λex313;λem360nm)	8-350 µg/l	2.7	55	[47]
Bromazepam	Tablets	Complexation reaction of bromazepam with iron(II) in HCl Spect. determ.*at (585 nm)	$5x10^{-4}$-$1.5x10^{-3}$mol/l	<1.2	36	[48]
Bromazepam	Tablets	Complexation with iron(II), Spect. determ.*at (585 nm) chemometric optimization	300-1100 mg/l	0.3	3	[49]

Calcium	Tablets; drinking water	Complexation with cresol-phthalein comlexone; Spect. determ.*at (578 nm)	0-20 mg/l	<1.4	43	[50]
Captopril	Tablets	Spect. determ.*at (400 nm)	$2x10^{-4}$-$1.4x10^{-3}$mpl/l	>2	60	[51]
Captopril	Tablets	Spect. determ.*at (535 nm)	20-1000mg/l	1.2	60	[52]
Ciprofloxacin	Tablets Infusion	Complexation with iron(III), Spect. determ.*at (447 nm)	50-500 mg/l	<0.9	60	[53]
Diclofenac	Tablets Injections Suppositories	Fluorimetric determination, (λ_{ex}305-95nm;λ_{em}430-70nm)	$1x10^{-5}$-$1x10^{-4}$ mol/l	<0.6	32	[54]
Doxycycline	Capsules; Tablets	Spect. determ.*at (213 nm)	2-100 μg/ml	0.5	6	[36]
Etilefrine hydrochloride	Pharmaceutical formulations	Spect. determ.*at (503 nm)	1-20 mg/l	<2.7	80	[55]
Fenoterol hydrochloride	Syrups	Spect. determ.*at (505 nm)	0.5-40mg/l	1.8	60	[56]

Indomethacin	Capsules; Tablets ext. release caps	Fluorimetric determination, (λ_{ex}350 nm;λ_{em}450nm)	$4x10^{-7}$-$1x10^{-5}$mol/l	<1.2	30	[57]
Iodide	Tablets; drops; Drinking water	Spect. determ.*at (410 nm)	0.002-0.5 mg/l	<3.6	15	[58]
Iodide	Tablets	Spect. determ.*at (600 nm)	0.1-6 μg/l	>2	80	[59]
Iron (III)	Capsules; Tablets	Spect. determ.*at (667 nm)	100-1000 mg/l	<1	8	[60]
	Pharm. products	Spect. determ.*at (667 nm)	0.30-80.00 mg/l	<0.8	30	[56]
Iron (II)	Pharmaceutical formulations	Spect. determ.*at (523 nm)	5.0-40.0 mg/l	3.0-4.0	100	[61]
	Tablets; caps; natural water	Spect. determ.*at (515 nm)	1-60 mg/l	<2.5	24	[62]
	Multi-vitamin Tablets	Spect. determ.*at (512 nm)	0.25-5.0mg/l	0.7-1.6	40	[63]
	Pharm. products	Spect. determ.*at (667 nm)	0.15-100.00 mg/l	<1.3	30	[64]

Isoxsuprine hydrochloride	Tablets	Spect. determ.*at (507 nm)	1-60 mg/l	1.4-1.6	60	[65]
Lisinopril	Tabs & caps	Fluorimetric determination, (λ_{ex}346;λ_{em}455nm)	0.3-10.00 mg/l	2	60	[66]
Norfloxacin	Tablets	Complexation with iron(III), Spect. determ.*at (430 nm)	50-400 ppm	<0.9	60	[53]
Magnesium	Tablets	Spect. determ.*at (570 nm)	0-2.0 mg/l	0.7-1.9	80	[67]
Metoclo-pramide	Tablets	Spect. determ.*at (495 nm)	13-130 µg/ml[§]	2.32	40	[68]
		Spect. determ.*at (495 nm)	3-42 µg/ml[¢]	2.78	18	[68]
Naproxen	Tablets	Fluorimetric determination, (λ_{ex}280;λ_{em}356nm)	$4x10^{-7}$-$1x10^{-5}$mol/l	<2.1	70	[69]
Oxybenzone	Milk; oil; lotions	Spect. determ.*at (376 nm)	840 µg/l	3.0-8.0	24	[70]
	Lipsticks	Spect. determ.*at (376 nm)	840 µg/l	6.0-12.0	24	[71]

Oxprenolol	Tablets	Oxidation with Ce(IV), Spect. determ.*at (480 nm)	50-400 ppm	0.88	120	[72]
Paracetamol	Tablets	Oxidation by hexacyanoferrate-(III) and reaction with phenol in the presence of ammonia, Spect. determ.*at (630 nm)	0-60 mg/l	1.2	27	[73]
	Tablets; suspensions; syrup	Spect. determ.*at (430 nm)	400-1000; 50-500 mg/l	3	60	[74]
			200-1000; 1000-2500mg/l	4	15	[74]
Perphenazine	Synthetic samples	Complexation with Pd(II), Spect. determ.*at (560 nm)	50-500 mg/l	<1	50	[75]
Phenylbenz-imidazole sulphonic acid	Sunscreen sprays	Spect. determ.*at (300 nm)	50-80 μg/l	1.0-6.0	20	[43]
Piroxicam	Capsules; Tablets	Fluorimetric determination, (λ_{ex}358;λ_{em}615nm)	0.1-1 ppm	>3.8	60	[76]

Procaine hydrochloride	Injections	Oxidation by permanganate chemiluminescence (≥390nm)	0.5-50 mg/l	<3.8	120	[42]
Promethazine	Tablets; elixir syrup	Complexation with Pd(II), Spect. determ.*at (540 nm)	50-400 mg/l	0.92	200	[77]
Promethazine	Tablets	Chemiluminescence (200-750)	$1.6x10^{-5}$-$1.9x10^{-3}$mol/l	0.75	180	[78]
Sulpha-nilamide	Standard solutions	Oxidation by permanganate chemiluminescence (≥390nm)	0.015-1.50 mmol/l	<2.3	120	[79]
Sulpha-cetamide	Eye drops	Oxidation by permanganate chemiluminescence (≥390nm)	0.012-1.20 mmol/l	<2.3	120	[79]
Sulphathiazole	Standard solutions	Oxidation by permanganate chemiluminescence (≥390nm)	0.009-0.92 mmol/l	<2.3	120	[79]
Sulpha-dimidine	Standard solutions	Oxidation by permanganate chemiluminescence (≥390nm)	0.035-0.87 mmol/l	<2.3	120	[79]
Sulpha-furazole	Tablets	Oxidation by permanganate chemiluminescence (≥390nm)	0.009-0.88 mmol/l	<2.3	120	[79]

Sulphametho-xypyridazine	Standard solutions	Oxidation by permanganate chemiluminescence (≥390nm)	0.019-0.95 mmol/l	<2.3	120	[79]
Sulpha-guanidine	Tablets	Oxidation by permanganate chemiluminescence (≥390nm)	0.010-1.00 mmol/l	<2.3	120	[79]
Tetracaine hydrochloride	Standard solutions	Oxidation by permanganate chemiluminescence (≥390nm)	0.2-25 μg/ml	<3.8	120	[42]
	Powder	Spect. determ.*at (572 nm)	25-300 μg/ml	2.07	40	[68]
Trimperazine	Synthetic samples	Complexation with Pd(II), Spect. determ.*at (515 nm)	50-400 ppm	<1	50	[75]
Trimethoprim	Tablets	Oxidation by permanganate chemiluminescence (370nm)	0.5-100 μg/ml	<1	120	[80]
Warfarin	Spiked samples	Fluorimetric determination, (λ_{ex}310;λ_{em}385nm)	0.1-1 μg/ml	1.5	180	[81]
Vitamin B_1	Multivitamins tabs & syrups; vit. B tabs	Fluorimetric determination, (λ_{ex}385;λ_{em}433nm)	0.06-8.0 μg/ml	1	30	[82]

Ascorbic acid (Vitamin C)	Tabs; capsules; Eff. tablets	Spect. determ.*at (510 nm)	20-300 ppm	<1.2	20	[83]
Ascorbic acid (Vitamin C)	Tablets; drops	Oxidation by Ce(IV) Spect. determ.*at (410 nm)	30-200 ppm	<1.2	120	[84]
Ascorbic acid (Vitamin C)	Tablets	Redox reaction between ascorbic acid and permanganate in acidic medium, Spect. determ.*at (525 nm)	0-1200mg/l	2.9	60	[85]
Ascorbic acid (Vitamin C)	Eff. Tablets; liquids	Absorbance of redox indicator ferroin incorporated into the membrane, (redox optical sensor- optode)	$2.5x10^{-3}$- $1x10^{-1}$mol/l	<2	17	[86]
		Chemiluminescence	$5x10^{-8}$-$1x10^{-5}$mol/l	<4	180	[87]
Zinc	Supplement, energy-boosting Tablets	Reaction with xylenol orange, Spect. determ.*at (568 nm)	10-60 mg/ml	<1	30	[88]

A review of SI techniques applied to pharmaceutical analysis [34]

Table 1.2 Electrochemical methods

Analyte	Matrix	Detection method	Linear range	R.S.D.%	Sample frequency h^{-1}	Ref.
Azidothymidine	Capsules	Amperiometric immunosensor, graphite paste with anti-AZT	80-2000 nmol/l	1	38	[87]
R-captopril	Spiked samples	Amperiometric biosensor	120-950 nmol/l	<0.1	34	[88]
			100-1000 nmol/l	<0.1	38	[89]
S-captopril	Spiked samples	Amperiometric biosensor	0.4-1.6 μmol/l	<0.1	34	[90]
	Tablets	Amperiometric biosensor	0.05-1.5 μmol/l	<0.2	80	[88]
	Spiked samples	Ion selective electrode	1-1000 μmol/l	<0.1	34	[89]
Captopril	Tablets	Ion selective electrode	$2x10^{-4}$-$1.4x10^{-3}$ mol/l	>1	5	[91]
Chloride	Pharmaceutical formulations	Optode	$1x10^{-3}$-1 mol/l	<1	14	[92]
		Ion selective electrode	$1x10^{-5}$-$1x10^{-2}$mol/l	<0.6	60	[92]
Clavulanate (Potassium)	Tablets	Ion selective electrode	$2x10^{-3}$-$1x10^{-1}$ mol/l	0.6	55	[93]
		Ion selective electrode	$2x10^{-3}$-$1x10^{-1}$ mol/l	0.5	53	[93]

Diclofenac	Tablets Injections Suppositories	Potentiometric detection, Ion selective electrode	$5x10^{-6}$-$1x10^{-2}$ mol/l	<0.4	33	[96]
S-enalapril	Raw material	Amperiometric biosensor	0.08-1.50ppm	<0.1	75	[97]
Penicillin-G	Pharmaceutical	Ion selective electrode	$2x10^{-4}$-$1x10^{-2}$mol/l	2	25	[98]
S-pentopril	Spiked samples	Amperiometric biosensor	0.2-6.0 µmol/l	<0.1	75	[97]
S-perindopril	Spiked samples	Amperiometric biosensor	60-800 nmol/l	<0.1	75	[97]
R-perindopril	Spiked samples	Amperiometric biosensor	60-900 nmol/l	<0.1	30	[97]
S-ramipril	Raw material	Amperiometric biosensor	0.12-0.60 µmol/l	<0.1	75	[97]
$_{L}$-thyroxine ($_{L}$-T4)	Tablets; injections	Amperiometric biosensor	10-780 ng/ml	<0.1	20	[99]
$_{D}$-thyroxine ($_{D}$-T4)	Tablets; injections	Amperiometric biosensor	50-500 nmol/l	<0.1	20	[99]

L-thriiodthyronine ($_L$-T3)	Raw material	Amperiometric biosensor	15-380 ng/ml	<0.1	20	[99]
Riboflavin (vitamin B_2)	Vitamin B tabs Multivitamin	Adsorptive strip. voltammetry determination and photodeg-radation study	0.0-0.8 μ mol/l	3	34	[100]
Vitamin B6	Multivitamin formulations	Ion selective electrode Potentiometric detection,	$1x10^{-4}$-$1x10^{-2}$ mol/l	<0.47	50	[101]

1.4 Automated process analysis in pharmaceutical production

The analytical determinations carried out in monitoring the chemical and biochemical reactions involved in the production process should systematically supply quantitative measurements. These determinations must eliminate, or at least minimize, sampling steps to give a rapid report on the state of a production process. For its robustness, capacity to collect samples directly from the reaction medium and a possibility of determining different species, the SIA technique was found to be adequate to control industrial processes. The SIA systems were also applied in the monitoring of the production processes of some drugs [102-105].

1.5 Automated drug dissolution and drug release

The dissolution assays of pharmaceuticals are very important for quality control of pharmaceutical products. Since these tests supply detailed information on the dynamic characteristics of the dissolution process, they are so useful for studies accompanying the development of new dosage forms. The automated SIA systems have the advantages of direct sample collection in the dissolution medium, carrying out parallel tests in the dissolution vessels and applying chemometric methods for the determination of multicomponents [106-109].

1.6 Functional cellular assays

Functional assays are designed to classify a drug as agonist or antagonist, depending on whether it induces or inhibits a biological response. If the binding of the drug to the receptor induces a cellular response, such response can be evaluated by monitoring suitable parameters like cytosolic Ca^{2+}, pH, acid release, lactate extrusion, glucose consumption, or oxygen consumption. Automation of the biological assays by the SIA technique using reagents in the heterogeneous SI-BI phase was proposed using various cell lines and measuring various evaluation parameters of the cellular response [110-113].

1.7 Chemometrics

Chemometrics is a chemical discipline that uses mathematics and statistics, so as to design or to select optimal experimental conditions, to provide maximum chemical information and to get good knowledge about chemical systems. [114,115].

1.7.1 Experimental design

1.7.1.1 Factorial design

Factorial designs were widely used in experiments where different factors were involved. It is necessary to study the overall effect of these factors on the response. In factorial designs, in each sample or replication of the experiment, all possible combinations of the factors levels are studied. The effect of a factor is defined as the change in response produced by the change in the level of the factor and is called the main effect and will be represented as the difference between the average response at the first level of the factor and the average response at the second level of the factor. The term effect interaction is used when the difference in response between the levels of one factor is not the same at all levels of other factors. The most elementary class of factorial design is 2^k factorial, where the base 2 stands for variables level and k for the number of factors studied. When one fits a first order equation to a 2^k factorial experiment, it is often likely to code the variables with -1 for the lowest level of a variable and +1 for the highest. Consider a 2^k factorial design matrix in terms of coded variables along with treatment combinations and their consequent responses (table 1.3) [116].

Table 1.3: treatment of coded factors and their responses

X_1	X_2	X_3	R
-1	-1	-1	a
-1	-1	+1	b
-1	+1	+1	c
+1	+1	+1	d
+1	+1	-1	e
+1	-1	-1	f
-1	+1	-1	g
+1	-1	+1	h

Where X_1, X_2 and X_3 are the factors studied and R is the response obtained. The treatment combinations order above may differ according to each individual design matrix run and the variables studied.

1.7.1.2 The ANOVA method

(ANOVA) or analysis of variance is a statistical technique used to analyze measurements depending on several simultaneously operating effects, and to know the importance of these effects and to estimate them. This analysis usually used to distinguish the sources of variability between laboratories, samples and replicates and to find out the influence of factors whether from humans, instruments or due to chemical factors on the results of chemical analysis. In ANOVA, one assumes that the errors are distributed, independent and having the same variance. ANOVA assumes that the variances of random variables, due to the effect of independent factors, are additive. Therefore, ANOVA can be used to break down the total variance into a sum of several components each corresponding to a source of variance [117].

1.7.2 Chemometric optimization [118-120]

The optimization of chemical systems using mathematical and statistical techniques in the chemical sciences has become widespread. It was used to relate some of the desired properties of materials to their composition. The desired properties are usually related to variables of composition by some functional mathematical form. Variables that affect the result interact in an unknown mathematical functional form and varieties of similar techniques are used to provide hints of the physical and chemical behavior of the system measurements to be used. Statistical and mathematical techniques are used to determine the importance of certain variables.

In order to apply mathematical results and numerical techniques of optimization to chemical problems, it is important to set out the boundaries of the system to be optimized. The key element in choosing a problem for optimization is the selection of the independent variables that are adequate for characterization of the system operating conditions. It is worthy to distinguish between the variables that can be treated and fixed and those that have great impact on the response. It is necessary to formulate the optimization problem with a single performance criterion.

After the performance criterion, boundary conditions and the independent variables have been selected, the coming step is to assemble a model that describes the manner in which the problems variables are related and the way in which the performance criterion is influenced by the independent variable. In general, optimization of chemical systems may be performed directly since the mathematical model that relates the independent variables to the performance criterion is unknown. Thus the independent variables of the system may be set to selected values, the system operated under those conditions and the system performance is evaluated using the observed performance. The method of optimization would be used to predict improved choices of the independent variables values.

1.7.3 The Simplex method

Optimization of technical systems is the process that can be implied to adjust the control variables and to find the levels, through which the best possible response can be achieved; different responses must be optimized concurrently. Optimization is usually done by "trial and error" or by changing one control variable while keeping the other variables constant. These methods are time consuming and require a lot of experiments to be carried. Simplex methods were introduced to overcome such problems.

The basic simplex method was introduced in 1962, by applying this method an optimum response could be achieved with fewer trials so that, it saves time and resources. In the modified simplex method, two major modifications were introduced to the original basic method that allows the expansion in the direction of favorable responses of contract than in the direction of unfavorable conditions. In the super modified simplex method the differences in the responses as the simplex vertexes are used to determine the vertex with a better response. The multisimplex method is a good example of implementation of the simplex optimization method. The user has a full control over the simplex optimization process and that is because of the pull-down menus. Interestingly enough, multisimplex has the ability to optimize several responses at the same time [121].

1.8 Chemometric optimization of the SIA methods

Chemometrics, in general, was introduced in analytical chemistry almost at the same time with FIA [119,120]. The most interesting is that chemometric approaches were used in some cases in the development and validation of FIA methods, since they can save time during the method development, and the detectors used with those systems can provide analytically useful data [115]. In FIA usually what will be optimized of response function is the peak height so as to increase the detectability of the system, reagent consumption, delay time and sample frequency [115].

Chemometrics was so successful for the optimization of chemical reactions in SIA procedures and was proven to be efficient enough when dealing with three or more interactive variables, as the utilized FI manifold or the chemical reaction-based assay were concerned.

Many papers in this subject (chemometric optimization of SIA methods for pharmaceutical analysis) were published. The first papers published were the assay of bromazepam [49], ascorbic acid [84], Perphenazine [122], flouroquinolone antibiotics [53], promethazine hydrochloride [163] and promazine [193]. The same group adopted chemometrical optimization FIA methods for some pharmaceuticals such as chlorpromazine [123], medazepam [124], and perphenazine [125], norfloxacin [126], promethazine hydrochloride [78, 127], procainamide hydrochloride [128], promethazine, chlorpromazine, trimeprazine [162], ciprofloxacin [129] and tetracycline [130].

1.9 Differential Electrolytic Potentiometry (DEP)

1.9.1 DEP historical background

Potentiometry is the field of electroanalytical chemistry in which potential is measured under the conditions of no current flow. The measured potential may then be used to determine the analytical quantity of interest, generally the concentration of some component of the analyte solution. Potentiometry is the most obvious method of finding the end point in titrimetric methods (134).

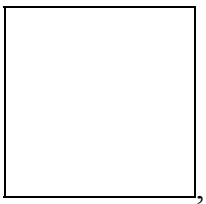
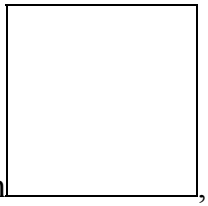
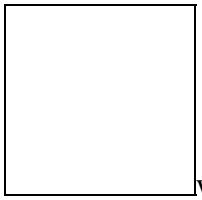
Electrolytic potentiotitrimetry is defined as the monitoring of the potential of a chemical cell with liquid junction during the course of a titration when a current of a few microamps is made to flow through the cell. The method is first proposed in by P. Dutoit and G. Weise (135). In order to avoid the possibility of deleterious effects which might be caused by repeated passages of current through the reference electrode, a third auxiliary electrode is used to conduct the current. The corresponding technique of applying a small current through a concentration cell without liquid junction during the course of titration is termed differential electrolytic potentiotitrimetry, and was introduced in 1922 (136). The recommended IUPAC nomenclature corresponding to the terms electrolytic potentiotitrimetry and differential electrolytic potentiotitrimetry are controlled current potentiometric titration and controlled current potentiometric titration with two indicator electrodes, respectively. Differential electrolytic potentiometry has the term electrolytic which was suggested by Bishop (137), implying that electrolysis is proceeding under the influence of a minute heavily stabilized current. This electrolysis current is less than the diffusion current of the electroactive species and the solutions are normally stirred.

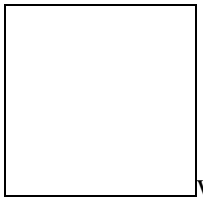
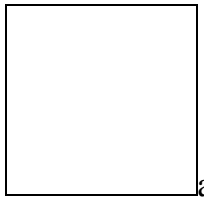
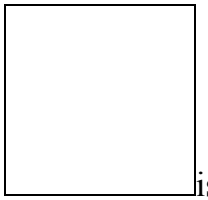
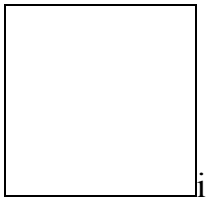
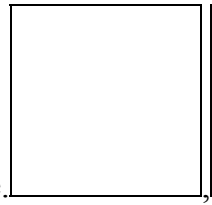
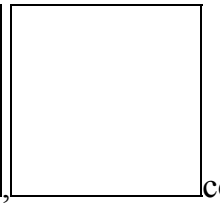
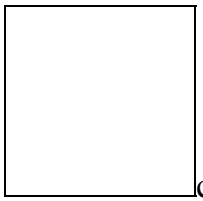
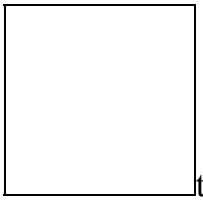
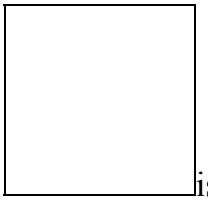
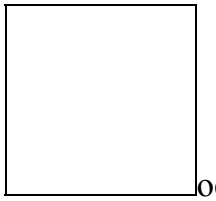
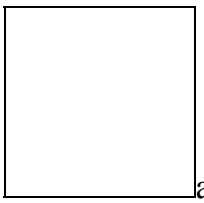
Basically, differential electrolytic potentiometry (DEP) as detection technique utilizes two identical metals that are polarized by a heavily constant current, and the potential difference between these indicator electrodes is measured. At the end-point, this potential difference produces a sharp symmetrical peak. DEP technique doesn't require a reference electrode and thus the difficulties of the salt bridge are eliminated. Moreover, polarization enhances

the response of the electrodes. This technique has been applied to various types of titrimetric reactions using different types of electrodes. Antimony electrodes have been used as indicating electrode for acid base reactions (138-141). Silver-silver halide electrodes are suitable for precipitation reactions (142-144). Platinum electrodes have been applied in oxidation-reduction reactions (145-147) and gold amalgam electrodes are appropriate for complexation reactions (148-150). DEP has been applied to various types of titrimetric reactions in non-aqueous media using different types of electrodes (151-155)

Polarization of an electrode is defined in the means of passing current through it. The potential of the electrode will be changed and it will be different from that of the zero-current potential. Depending on the type of polarization the anodic polarization will make the potential of the electrode more positive than the zero current. In the case of cathodic polarization, the electrode will attain more negative potential than the zero current electrodes. The difference in potentials is due to the overpotential.

Overpotential

In the absence of net current flowing in the external circuit the sum of cathodic and anodic current is equal to zero i.e.  , io represents the exchange current that flows continuously in the compact layer. When  , the electrode potential will have the equilibrium zero-current value,  with respect to saturated hydrogen electrode.

When a net current is passing through the electrodes its potential will have a new value,  which is the potential of the working electrode. The difference between  and  is called the overpotential  i.e.  ,  consists of three components,  charge transfer or activation overpotential,  the mass transfer or concentration overpotential and  is the resistance potential,  occurs due to the slowness of the electrode process, depending upon the nature of electrode surface, its surface conditions and the concentration of electroactive species present in the medium,  arises when the passage of current causes a net reaction at the electrode surface and independent of the nature of electrode. This results in a difference in concentrations of reactants and products at the electrode surface

from those in the bulk of solution forming concentration gradients. Hence reactants migrate towards and products migrate away from the electrode.

As a result of the overpotential which is due to the polarization process, a potential difference will develop between the two electrodes. The anode will lead the zero-current electrode and the cathode will lag the potential of that electrode. Hence ΔE which is the difference between the potentials of the anode and that of the cathode will be measured during the course of the reaction. Finally, a plot between ΔE and the volume being added from the burette will be established and the end- point is calculated from this plot by considering the tip of the resulting peak. Different parameters such as the value of the current, the stirring rate, and the types of the electrodes and the geometry of the cell have to be optimized in order to get a symmetrical peak with a considerable height.

The shapes of titration curves resulting from two identical indicator electrodes polarized at constant current are characteristics of the reversibility of both the titrant and titrand, because reversible couples produce a current-potential curve whose reciprocal slope dE/di in the vicinity of zero current is relatively small. The maximal e.m.f. between the two electrodes at the equivalence point is approximately equal to the difference between the formal potentials of the two couples (curve 1 in Fig.1.6). If a titrand which establishes a reversible couple is titrated with a titrant which doesn't function reversibly, the e.m.f. between the two identical polarized electrodes will follow the course indicated by (curve 2 in Fig.1.6). The current potential curve of the irreversible titrand couple will have a large value of the reciprocal slope dE/di at zero current as shown in curve 3 in Fig.1.6.

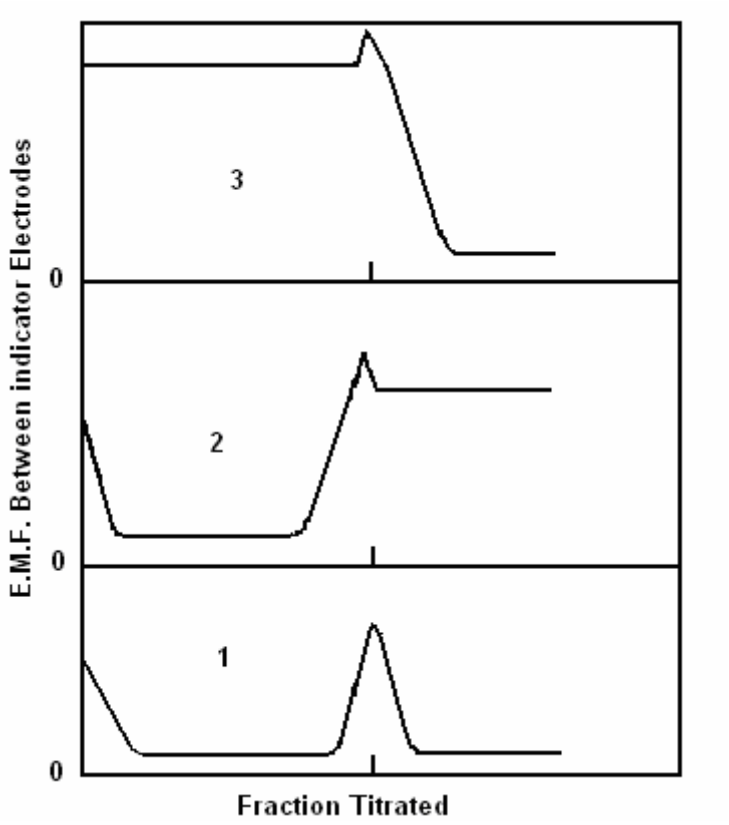


Figure 1.6: Types of titration curves with two identical indicator electrodes polarized at constant current. (1) titrand and titrant couples both reversible. (2) Titrand couple reversible, but titrant couple irreversible. (3) Titrand couple irreversible, but titrant couple reversible.

The use of polarized electrodes can entail an error corresponding to the amount of electrolysis at the electrode. This is always present with a single polarized electrode, and also occurs with two identical polarized electrodes whenever the reaction couple behaves irreversibly that occurs with two identical electrodes. The error can be reduced to negligible proportions by using small electrodes and a small electrolysis current. The main value of polarized indicator electrodes is for irreversible couples where an un-polarized electrode is often very slow in acquiring a constant potential whereas a polarized indicator electrode reaches a steady potential quickly at constant current, and large variations of potential are observed at the end point.

1.10 Objectives

1.10.1 Objectives of the SIA method

This study was conducted to fulfill the following objectives:

1- To perform a comprehensive procedure for the oxidation of both chlorpheniramine maleate and verapamil hydrochloride by permanganate in sulphuric acid media utilizing SIA.

2- To construct SIA manifolds suitable for the method of assay of chlorpheniramine maleate and verapamil hydrochloride.

3- To establish appropriate SIA programs for controlling the newly adopted methods.

4- To search for the optimum conditions for the assay methods.

5- To conduct a chemometric optimization for the methods under study.

6- To evaluate the linearity, accuracy, precision, repeatability, robustness and cost of the newly adopted method.

7- To validate the newly adopted method by comparison with the BP standard method.

1.10.2 Objectives of the DEP method

This study was performed to fulfill the following objectives:

1- To perform a comprehensive study for the oxidation of promethazine hydrochloride with Cerium (IV) in acidic medium and its applicability to electrochemical detection.

2- To construct a system suitable for electrochemical detection.

3- To search for the optimum conditions for the assay method.

4- To establish a computer program that controls the syringe pump for the addition of appropriate volumes.

5- To evaluate the accuracy, precision, repeatability, robustness and cost of the newly adopted method.

6- To validate the newly adopted method by comparison with the BP standard method.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Instrumentation

2.1.1 SI analyzer

The SI analyzer manifold used in this work consisted of a sequential injection analyzer combined with a fiber optic spectrophotometer (fig 2.1). The SI analyzer system is an Alitea USA/FIALab 3000 (Medina, WA USA). The following sections present descriptions and specifications of the components of the SI analyzer system

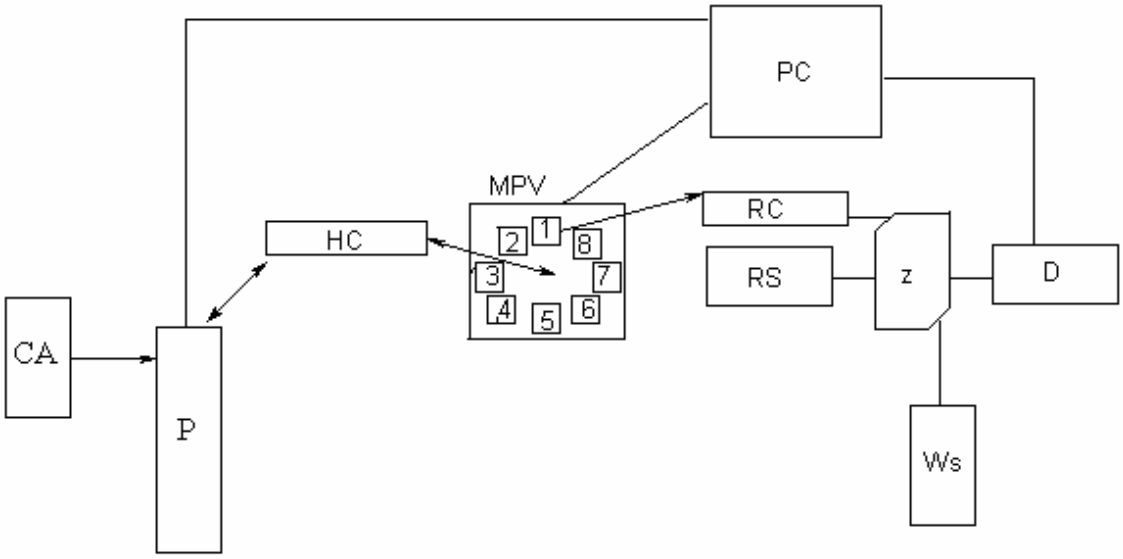


Fig. 2.1: Sequential injection analyzer manifold diagram, CA: carrier (water); P: syringe pump; HC: holding coil; MPV: multi-position valve; 1-8: ports; RC: reaction coil; RS: radiation source; Z: Z-flow cell; D: detector; PC: personal computer; Ws: waste.

2.1.1.1 Syringe pump (P)

Only one syringe pump is needed because SIA is operated as a single line system. The pump is operated in an intermittent forward and reverse mode. The 24,000-step stepper motor driven syringe pump (with an optical encoder feedback and 1.5 seconds to 20 minutes per stroke) was connected to a multi-position valve through a reaction coil. The glass syringe pump is interchangeable

with great ease and available in size from 100 micro liters up to 10 milliliters to allow aspiration of adequate volumes of carrier, to deliver solutions and to flush the system. The volume of the syringe pump used is 2500 micro liters.

2.1.1.2 Multi-position valve (MPV)

The MPV used has eight ports with standard pressure of 250 psi (gas)/600 psi (liquid); zero dead volume. It is chemically inert. Port selection is controlled automatically through a software program.

2.1.1.3 Pump tubing

It is a 0.30,, ID Teflon type supplied by Upchurch Scientific, Inc. (Oak Harbor, WA USA). It is used for the connection of the different units of the SIA manifold and for making coils.

2.1.1.4 Coils

Coils were manually prepared by coiling a pump tubing of 0.30" ID Teflon type. The following sections describe the coils of the SIA manifold.

2.1.1.4.1 Holding coil (HC)

The HC was placed between the central port of the MPV and the P. The aspirated solutions are held in this coil. Other processes like mixing, dilution and reaction take place. The mixture is dispensed directly to the Z-flow cell or through the reaction coil which is optional according to the type of reaction. The length of the pump tubing of the HC used in this work was 200 cm.

2.1.1.4.2 Reaction coil (RC)

Some reactions applied to measurements require different conditions such heating, increasing delay time of reaction. In such cases an additional coil was added, and called the reaction coil (RC). The RC usually placed between

MPV and the Z-flow cell. The RC is helpful in kinetic-based reactions. The length of the pump tubing of the RC used in this work was 160 cm.

2.1.2 Miniaturized spectrometer

The fiber optic spectrometer is composed of a light source, fiber optic connectors, Z-flow cell, windows and a detector. Specifications, descriptions and set up of the optic system are discussed below.

2.1.2.1 Fiber optic SMA Z-flow cell

It is a 10 mm path length plexiglass compatible with standard SMA terminated fiber optic and contains fused silica windows as wetting surfaces at each fiber optic junction. The SMA Z-flow cell is designed to accommodate common optical measurements and to minimize bubble trapping in flow injection and sequential injection analysis.

2.1.2.2 Windows

The windows used in this study are a UV fused silica grade. It is a hard and chemically resistant material which has a high UV through IR transmittance (170 nm through ~2000 nm). The windows are removable for cleaning or replacement with another material that has different optical band-pass properties.

2.1.2.3 Radiation source

The radiation source used is a LS-1 tungsten halogen with the following specifications:

Spectral range:	360 nm -2μm
Dimensions:	9.0 cm × 5.0 cm × 3.2 cm
Power output:	6.5 watts
Power input:	12 VDC/800 mA
Bulb life:	900 h
Bulb color temp.:	3100 K
Time to stabilize output:	~ 30 min

Stability:	after 100-h burn-in, decay rate is ~ 0.1%h
Internal filter:	BG-34 conversion filter
Connector:	SMA 905

2.1.2.4 Detector

The USB2000 fiber optic spectrometer was manufactured by Ocean Optics® and it was used in this work. The name and function of each component are presented in table 2.1. The following points describe the specifications of the spectrometer.

Dimensions:	8.91 mm × 6.33 mm × 3.44 mm
Detector range:	200 – 1100 nm
Gratings:	14 gratings; UV through shortwave NIR
Entrance apertures:	5, 10, 25, 50, 100 or 200 mm wide slits or fiber (no slits)
Optical resolutions:	~0.3 – 10.0 nm FWHM
Dynamic range:	2 × 108 (system); 2000:1 for a single scan
Stray light:	<0.05% at 600 nm; <0.10% at 435 nm; <0.10% at 250 nm
Sensitivity: (estimate)	400nm – 90 photons/count; 600 nm – 41 photons/count; 800 nm – 203 photons/count
Fiber optic connector:	SMA 905 to single-strand optical fiber (0.22 NA)
Data transfer rate:	Full scans into memory every 13 milliseconds
Integration Time:	3 milliseconds to 65 seconds

2.1.3 Conventional spectrophotometer

The UV/VIS spectrophotometer provided by Perkin Elmer, Lambda EZ210 was used for applying the BP standard methods.

2.1.4 Oscilloscope

The oscilloscope provided by Tektronix model 2213A 60 MHz to show the frequency and square wave for both AC and DC current generated by the circuit.

Table 2.1 Functions of the USB2000 fiber optic spectrometer

Item	Name	Function
1	SMA connector	Light from the input fiber enters the spectrometer through this connector.
2	Slit	Regulate the amount of light that enter the spectrometer and controls spectral resolution.
3	Filter	Restricts the optical radiation to pre-determined wavelength regions.
4	Collimating mirror	Focuses the entering light towards the grating.
5	Grating	Diffracts light from the collimating mirror and directs the diffracted light onto the focusing mirror
6	Focusing mirror	Receives light reflected from the grating and focuses the light onto the CCD detector and L2 detector collection lens.
7	L2 Detector collection lens	Attaches to the CCD detector. It focuses light from a tall slit onto the shorter CCD detector elements.
8	CCD Detector	Collects the light received from the focusing mirror or L2 detector collection lens and converts the optical signal to digital signal.

2.1.5 Voltmeter

A digital multimeter provided by Agilent model 34401A to measure the differential electrolytic potential between the two electrodes.

2.1.6 Microameter

This microameter is provided by Simpson co. model no. 374 to measure the current that polarizes the electrodes.

2.1.7 Potentiometer

A potentiometer provided by MeasureNet Co. USA was used for applying the BP standard methods.

2.1.8 Electronic circuit

An electronic circuit was designed to generate an AC and DC current for the polarization of electrodes and the consequent measurement of the potential difference between them by a voltmeter attached to the unit as in figure 2.6.

2.1.9 Platinum electrodes

Two identical platinum electrodes were employed, and polarized by the passage of current through them, and therefore change of their potential from that at zero current.

2.1.10 Glass cell

A specific glass cell was designed with openings from the top; one slit is for the insertion of the Teflon coils and the other two for the electrodes. In this cell the reactants were mixed together and where electrolysis takes place.

2.1.11 Programmable syringe pump (SP)

A computer controlled programmable syringe pump supplied from J-Kem, Scientific USA was used in the differential electrolytic potentiometric titration for promethazine hydrochloride to deliver the exact required volumes of the reactants in microliterers.

2.1.12 Magnetic stirrer and magnets

An electronic magnetic stirrer and small magnets were used for proper mixing of the reactants.

2.2 Software packages

2.2.1 FIALab®

The FIALab for windows version 5.5 from FIALab® (Medina, WA USA) was utilized for controlling the SIA system.

2.2.2 OOIbase®

Spectrophotometric data acquisition and treatment were performed by 2.2.2 OOIbase® software version 2.0.1.2, driver version 4.07.00, 2002 supplied from Ocean Optic, Inc. The software calculates the absorbance (Aλ) at wavelength λ using equation 2.1.

$$A\lambda = -\log_{10}\left[\frac{S\lambda - D\lambda}{R\lambda - D\lambda}\right] \qquad (2.1)$$

Where Sλ is the sample intensity at the wavelength λ; Dλ is the dark intensity at the wavelength λ; Rλ is the reference intensity at the wavelength λ.

2.2.3 SigmaPlot®

SigmaPlot® supplied from Jandel Sceintific Corporation (1994) version 1.02 for windows 3.95 enhanced modes was used for chemometric calculations and for drawing of the three dimensional graphs.

2.2.4 Excel®

Excel® for windows 2003 was used for plotting calibration curves and their related calculations.

2.3 Chemicals and Reagents

2.3.1 Promethazine hydrochloride

Promethazine hydrochloride supplied by sigma P4651 was used for preparing 0.001 mol 1^{-1}as primary standard solution. The primary standard solution and the working standard solutions were immediately prepared before use.

2.3.2 Chlorpheniramine maleate

Chlorpheniramine maleate supplied by sigma C3025 was used for preparing 200 ppm as primary standard solution. The primary standard solution and the working standard solutions were immediately prepared before use.

2.3.3 Verapamil hydrochloride

Verapamil hydrochloride supplied by sigma V4629 was used for preparing 200 ppm as primary standard solution. The primary standard solution and the working standard solutions were immediately prepared before use.

2.3.4 Potassium permanganate

Potassium permanganate from Fisher Scientific Co. Fair Lawn, New Jersey USA was used to prepare a stock solution of 0.01mol 1^{-1}. The stock solution was stored in a dark place and covered with aluminum foil and weekly standardized by sodium oxalate. Working solutions were prepared daily.

2.3.5 Cerium (IV) ammonium sulphate

Ammonium cerium sulphate-2-hydrate,CeNH4)4(SO4)4. 2H2O from BDH Chemicals LTD, Poole, England was used to prepare 0.1 mol 1^{-1} in 0.01 mol 1^{-1} sulfuric acid. Standard working solutions were prepared after standardization of stock solution by sodium oxalate.

2.3.6 Sulfuric Acid

95-98 % (W/V^{-1}), 1.84 g 1^{-1}, supplied from Fisher Scientific Co., Fair Lawn, New Jeresy USA was used to prepare working standard solutions.

2.3.7 Hydrochloric Acid

31-32 %, Specific gravity 1.16 g 1^{-1}, from BDH Chemicals LTD, Poole England was used to prepare working standard solutions.

2.3.8 Sodium hydroxide

97% sodium hydroxide supplied from Montplet & Steban SA, Barcelona, Spain was used to prepare 1 mol 1^{-1} stock solution. Standard working solutions were prepared and standardized by potassium hydrogen phthalate.

2.3.9 Nitric Acid

64-66% nitric acid, 1.4g 1-1, from Fluka Chemie AG, CH-9470 Buchs and packed in Switzerland was used to prepare Aqua Ragia

2.3.10 Perchloric Acid

Perchloric acid supplied from Fluka Chemie AG, CH-9470 Buchs and packed in Switzerland was used in standard potentiometric titration for chlorpheniramine.

2.3.11 Ether

Ether supplied from BDH Chemicals LTD, Poole England was used in the official method for the determination of chlorpheniramine tablet form.

2.4 Pharmaceutical samples

To validate the newly adopted method, some of the available pharmaceutical formulations containing the drug under study were collected from drug stores. The SIA, DEP and BP standard methods were applied to the collected samples.

2.5 Procedure

2.5.1 Reagents and samples preparations

Standard and reagent solutions were prepared in distilled water. Blank solutions were prepared in the same way as for that of working solutions. Tablets were prepared by triturating 20 tablets, and then the required amount of powder was dissolved in water and filtered. The filterate was diluted to the required volume.

2.5.2 Parameters of the USB2000 fiber optic spectrometer

The following sections describe the spectrometer setup adjusted for all measurements performed in the SIA work [131].

2.5.2.1 Integration time

Integration time represents the CCD charge time that will elapse before the A/D conversion. A time of 5 milliseconds was used throughout this study and gave acceptable results. Usually maximum signal is obtained by increasing integration time.

2.5.2.2 Detector to average

It describes many adjacent CCD pixel signals that are to be added together for a boxcar average, which will be assigned to a single A/D value. Increasing the number of detector to average loses the overall resolution of spectrum, but it decreases the noise of a spectrum. There were three levels 1, 3 and 5. The medium level of detector to average 3 was the one to be used.

2.5.2.3 Sample to average

Sample averaging will average several spectra to produce an average spectrum over a given period of time. Its advantage is to reduce the noise by square root of the number of averages without affecting the spectral resolution, but it may affect temporal (time) resolution and may lower sampling rate.

2.5.2.4 Sample rate

Sample rate is the rate of spectra acquisition and it was adjusted to 4 Hz. An ideal rate of 4-Hz collects spectra every 250 ms. In practice, the actual sampling rate may be significantly slower than what is selected when high integration time is used. In most cases, 4 to 8 Hz is the maximum.

2.5.3 Preparation of SI analyzer manifold

2.5.3.1 Fiber optic spectrometer setup

Usually, spectrometer setup should not be changed unless instrumental malfunctioning took place. However before starting analysis, some parameters should be adjusted as shown in figure 2.2. The following sections describe the spectrometer setup and those parameters could be adjusted.

2.5.3.1.1 Dark baseline

Prior to data acquisition and before switching on the lamp, to reset all setup parameters and take a dark baseline, the spectrometer was logon and concomitantly a dark scan was performed. Figure 2.3 shows the line of the dark scan.

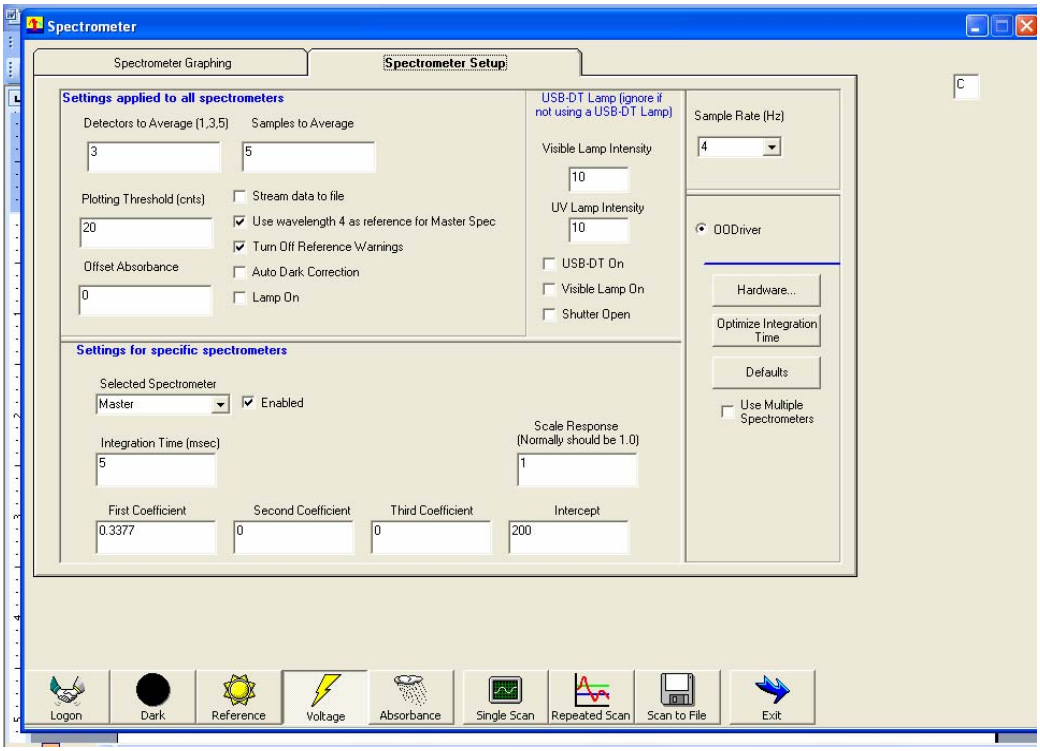


Figure 2.2: Parameters of USB2000 fiber optic spectrometer adjusted for the current work

2.5.3.1.2 Reference scan

After updating a dark background, to get a raw light input, the reference scan was performed after putting on the lamb and allowing it to warm for 30 minutes. In most cases the reference scan gives spectrum as shown by figure 2.4. The ideal reference scan should be not less than 500 and not greater than 4095

2.5.3.2 Washing the channels of SI analyzer

Before loading solutions to the SIA system, the syringe, the main channel, holding coil, reaction coil and tubes connected to the MPV were washed with water. A fully automated program was written to control the washing process. The program is presented in appendix 1.

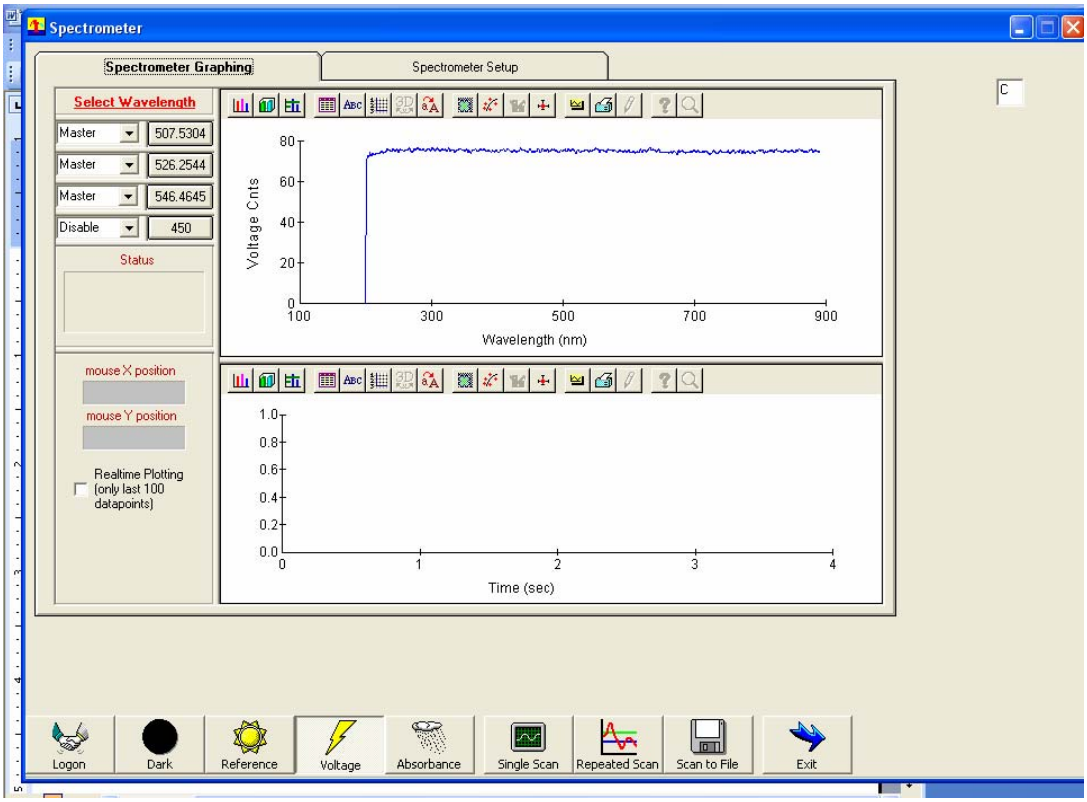


Figure 2.3: Spectrometer dark background

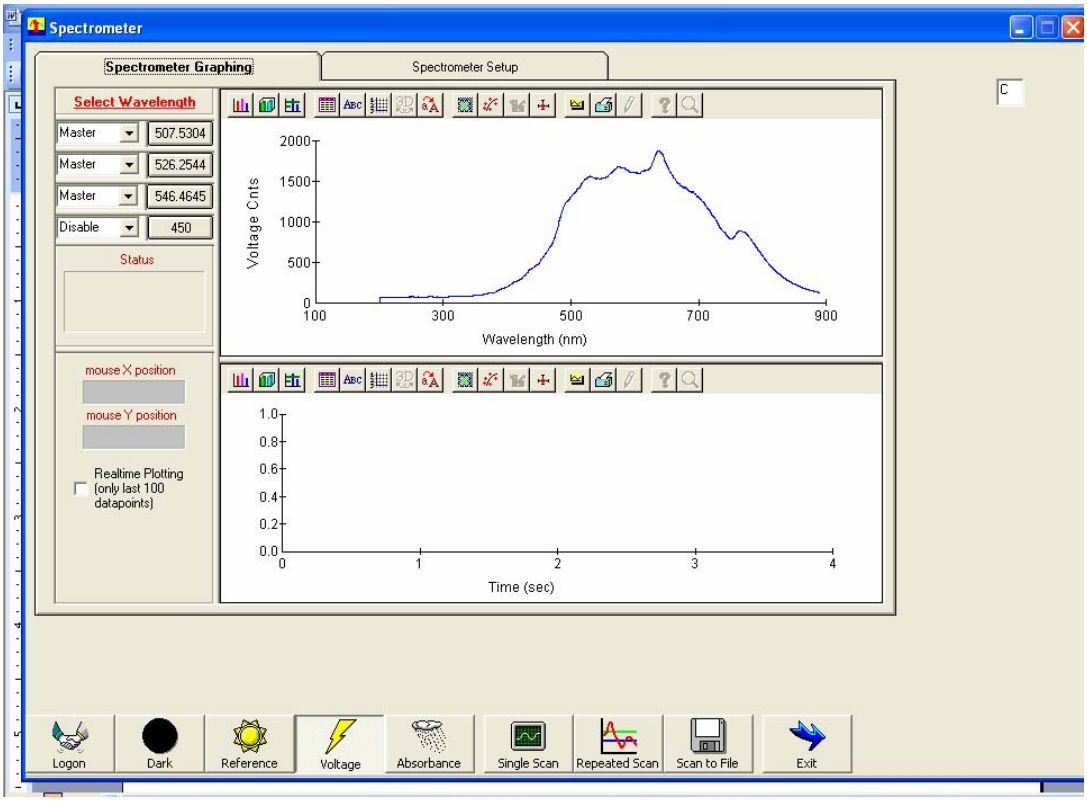


Figure 2.4: Spectrometer reference background

2.5.4 The BP standard methods

To validate the newly adopted methods (SIA and DEP) the BP standard methods were applied for the assay of pharmaceuticals under study.

2.5.4.1 Promethazine hydrochloride tablets analysis

20 tablets were weighed and powdered. A powder containing 50 mg of promethazine hydrochloride was dissolved in 10 ml of 2 mol l^{-1} hydrochloric acid and then 200 ml of water was added. The mixture was shaken for 15 minutes and sufficient volume of water was added to produce 500ml. 50ml of the mixture was centrifuged. 10 ml of 0.1 mol l^{-1} hydrochloric acid was added and sufficient volume of water was added to produce 100 ml. The absorbance of the resulting solution was measured at 249 nm.

2.5.4.2.1 Chlorpheniramine tablet analysis

20 tablets were weighed and powdered. A powder containing 3 mg of chlorpheniramine was shaken with 20 ml of 0.05 mol l^{-1} sulfuric acid for 5 minutes 20 ml of ether was added and shaken, the acid layer was filtered. The ether layer was extracted with two 10 ml quantities of 0.05 mol l^{-1} sulfuric acid. Each acid layer was filtered and washed with 0.05 mol l^{-1} sulfuric acid. The combined extract and the washings were made just alkaline to litmus with 1 mol l^{-1} sodium hydroxide, 2 ml in excess of sodium hydroxide were added and the mixture was extracted with two 50 ml quantities of ether. The ether extract was washed with 20 ml of water and with 20, 20 and 5 ml of 0.25 mol l^{-1} sulfuric acid respectively. The combined extracts were diluted to 50 ml with 0.25 mol l^{-1} sulfuric acid, then 10 ml was diluted to 25 ml with a 0.25 mol l^{-1} sulfuric acid. The absorbance of the resulting solution was measured at 265 nm.

2.5.4.2.2 Chlorpheniramine injection analysis

A volume of injection containing 10 mg chlorpheniramine was diluted to 500 ml with 0.25 mol l^{-1} sulfuric acid. The absorbance of the resulting solution was measured at 265 nm.

2.5.4.3.1 Verapamil tablet analysis

20 tablets were weighed and powdered. A powder containing 100 mg of verapamil hydrochloride was shaken with 150 ml of 0.1mol l^{-1} hydrochloric acid for 10 minutes. Sufficient volume of 0.1 mol l^{-1} hydrochloric acid was added to produce 200 ml and then the mixture is filtered. 10 ml of the filterate was diluted to 100 ml with water. The absorbance of the resulting solution was measured at 278 nm.

2.5.4.3.2 Verapamil injection analysis

To a volume containing 5 mg of verapamil hydrochloride, 100 ml of 0.01mol l^{-1} hydrochloric acid was added. The absorbance of the resulting solution was measured at 278 nm.

2.6 Promethazine Hydrochloride Assay

2.6.1 Introduction

Promethazine hydrochloride is chemically named as 10-N, N-dimethyl-1-(10H-phenothiazine-10-yl)propan-2-amine hydrochloride or 10H-phenothiazine-10-ethanamine, N, N, a-trimethyl-,monohydrochloride (figure 2.5). It is white or faintly yellowish crystalline powder, very soluble in water, freely soluble in alcohol and in methylene chloride [132]

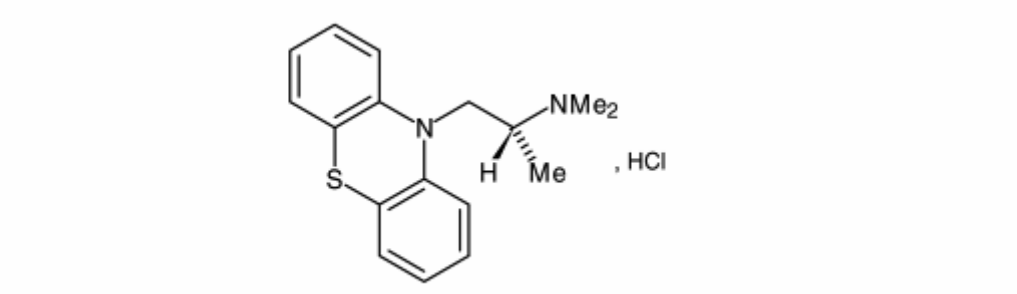


Figure 2:5 Promethazine hydrochloride structure

Phenothiazines form a large class of basic drugs used primarily for the treatment of moderate to severe mental disorders. They can also be used as anti-emetics, sedatives, antipruritics, antidyskinetics, analegesics and antihistaminics [134]. Promethazine hydrochloride is one of the most important drugs of the phenothiazine group. As an antihistamine with H_1-receptor antagonism promethazine can be used for the symptomatic relief of hypersensitivity reactions [132, 135]. Promethazine hydrochloride is usually available as mono component in dosage forms involving injections, oral suspensions, tablets, suppositories and syrup [132, 133].

2.6.2 Assay methods of promethazine hydrochloride in pharmaceutical formulations

Many analytical methods for the assay of promethazine hydrochloride in pharmaceutical preparations were established. Several methods were proposed involving official standard methods as well as non-official standard methods. The sections below describe the advantages and disadvantages of some proposed methods.

2.6.2.1 Official standard methods

The BP [132] method for the assay of promethazine hydrochloride in the generic form is a potentiometric titration with sodium hydroxide after dissolving the sample in ethanol and hydrochloric acid. A direct spectrophotometric method is provided for the injection form, the absorbance is measured at wavelength 249 nm after a series of dilutions with hydrochloric acid. For oral solution form, sodium hydroxide is added to the sample, shaken for 1 minute, the mixture is extracted with chloroform and the combined extract is evaporated to dryness at 30ºC under pressure 2 kPa. The residue is dissolved in hydrochloric acid and diluted with water. The absorbance is measured at wavelength 336 nm. It is clear that in this method lengthy extraction processes were applied and it consumes time and resources. The tablet form is assayed in the same way as the injection form; the sample is triturated with 2mol l^{-1} hydrochloric acid and shaken well for 15 minutes and then centrifuged and diluted with 0.1 mol l^{-1} hydrochloric acid, the absorbance is measured at wavelength 249nm. This method is lengthier compared with that of the injection form.

In the USP monograph [133], promethazine hydrochloride generic form is assayed by titration with perchloric acid in the presence of glacial acetic acid and mercuric acetae using crystal violet as an indicator. For injection form an HPLC method is used. The mobile phase is composed of sodium 1-pentanesulfonate, acetonitrile and glacial acetic acid. The method involves filtration and degassing which imply tedious sample treatments; in addition to that it is a lengthy procedure. Spectrophotometric method is provided for suppositories, the

palladium reagent is prepared by dissolving palladium chloride in hydrochloric acid and the mixture is warmed in a steam bath and diluted slowly. To part of the palladium reagent solution, sodium acetate is added and the pH is adjusted. The melted sample is dissolved in hexane and then hydrochloric acid is added. Promethazine hydrochloride is extracted by hydrochloric acid, shaken, diluted and finally filtered. The absorbance of the solution is measured at wavelength 450nm. Spectrophotometric method of analysis is provided for the oral solution and syrup forms of promethazine hydrochloride, the sample is alkalized to promethazine base and extracted by chloroform, the combined chloroform extract is then washed by hydrochloric acid and evaporated on a steam bath with the aid of air current. The residue is warmed and dissolved in sulfuric acid, cooled and diluted by sulfuric acid and filtered. The absorbance of the resulting solution is measured at wavelength 289 nm.

2.6.2.2 Other non-official methods

There are many methods adopted for the assay of promethazine hydrochloride in pharmaceutical preparations including titrimetric [134,135], spectrophotometric [136-144], flourimetric [145-148], and chemiluminescence [149].In addition to high performance liquid chromatography [150,151], voltammetric [152,153], potentiometric [154,155], electrophoresis [156-159] and coductimetric [160]. Recently, flow injection analysis combined with different detectors including spectrophotometry [75, 161-165], chemiluminescence [79, 166], spectroelectoanalysis [167] and turbidimetry [168] have been extensively studied for the assay of promethazine hydrochloride in pharmaceutical products.

Many of the above mentioned methods were indirect, with narrow linearity and employing conventional spectrophotometric techniques. Others are lengthy.

2.6.3 Construction and programming of electrochemical system

A suitable electrochemical system was constructed (figure 2.6) for the purpose of performing a newly adopted method for the assay of promethazine

hydrochloride. For the oxidation of promethazine by cerium IV in acidic medium, a suitable program was written to control the whole process. The system involves the employment of a programmable syringe pump to deliver the exact volumes into the glass cell where the electrolysis occurs by the aid of polarized identical platinum electrodes. The polarization of the electrodes happens when a minute current passes through them; the current was generated by the electronic circuit and measured by the microameter. The voltammeter was used to measure the potential difference between the two electrodes and the oscilloscope to show the status of the current whether it is a DC or an AC.

The program controlling the assay of promethazine hydrochloride is simple and as follows:

1. The system was set as shown in figure 2.6
2. The syringe pump was homed
3. Each reagent was connected to a specified port; port 1 for promethazine, port 2 for the acid and port 3 for CeIV.
4. A known volume in microliters of promethazine was first delivered into the glass cell, and then the supporting electrolyte which was sulfuric acid was added and stirred with magnetic stirrer and then titrated against CeIV.
5. The endpoint was detected potentiometrically.

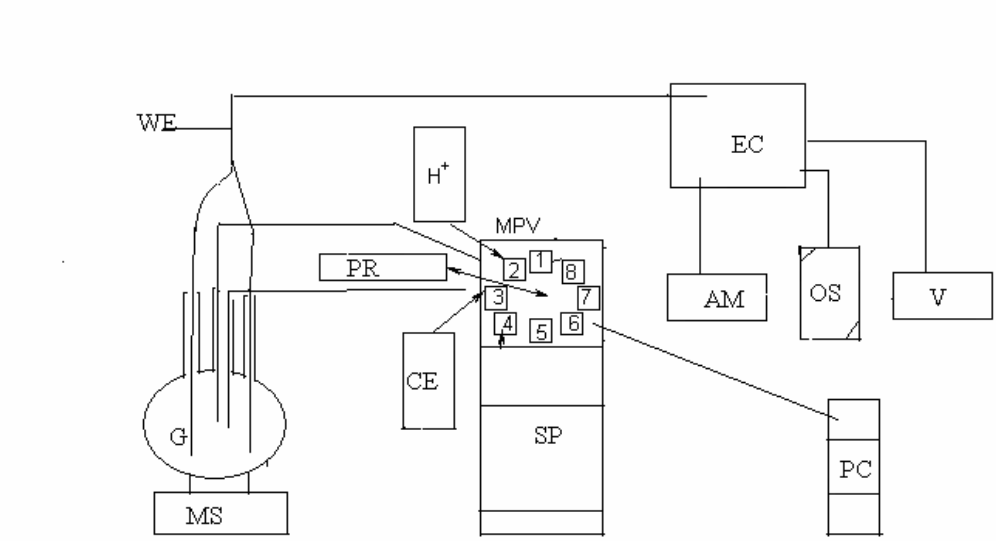


Figure 2.6: Electrochemical system; EC: electronic circuit; V: voltmeter; OS: oscilloscope; AM: microameter; PC: personal computer; SP: programmable syringe pump; MPV: multiposition valve; H: sulfuric acid; Ce: ceriumIV; PR: promethazine hydrochloride; WE: wire with two electrodes; G: glass cell; MS: magnetic stirrer.

2.7 Chlorpheniramine maleate Assay

2.7.1 Introduction

Chlorpheniramine maleate is chemically named as 3-(4-chlorophenyl)-N-N-dimethyl-3-pyrid-2-ylpro-panamine hydrogen (2)-butenedioate (figure 2.7). It is a white, crystalline powder, freely soluble in water, soluble in alcohol and slightly soluble in ether [132]

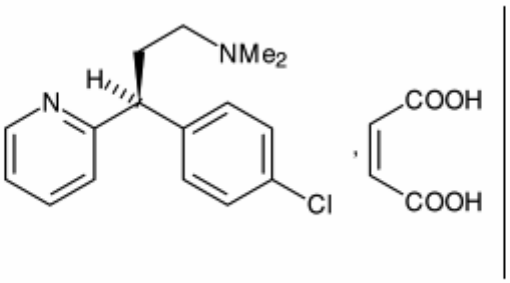


Fig. 2.7 Chlorpheniramine maleate structure

Chlorpheniramine maleate, an alkylamine derivative, is a sedating antihistamine that causes a moderate degree of sedation; it also has antimuscarinic activity. It is used for the symptomatic relief of allergic conditions including urticaria and angioedema, rhinitis and conjunctivitis, and in pruritic skin disorders. It is a common ingredient of compound preparations for symptomatic treatment of coughs and common cold [169].

2.7.2 Assay methods of chlorpheniramine maleate in pharmaceutical formulations

Many analytical methods for the assay of chlorpheniramine maleate in pharmaceutical preparations were established. Several methods were reported involving official standard methods as well as non-official standard methods. The sections below describe the advantages and disadvantages of some methods.

2.7.2.1 Official standard methods

The BP [132] method for the assay of chlorpheniramine maleate in the generic form is a potentiometric titration with perchloric acid after dissolving the sample in anhydrous acetic acid. A direct spectrophotometric method is provided for the injection form, the absorbance is measured at wavelength 265 nm after dilution with 0.25 mol l^{-1} sulphuric acid. For oral solution form, gas chromatography is employed to determine the content of chlorpheniramine maleate in the sample. For the tablet form 0.05 mol l^{-1} sulphuric acid is added to the sample, shaken for 5 minutes, then ether is added to the mixture. The acid layer is then filtered; the ether layer is extracted with two quantities of 0.05 mol l^{-1} sulphuric acid and then filtered and washed with more 0.05 mol l^{-1} sulphuric acids. The combined acid washings and extracts are made just alkaline to litmus paper by adding 1 mol l^{-1} sodium hydroxide, then 2 ml of sodium hydroxide are added in excess. This is followed by extraction with two quantities of ether and washing of each extract with water followed by successive extraction with three quantities of 0.25 mol l^{-1} sulphuric acid and a series of dilutions with more 0.25

mol l^{-1} sulphuric acid. The absorbance is measured at wavelength 265 nm. It is clear that in this method a lengthy extraction procedure is followed.

2.7.2.2 Other non-official methods

Several analytical methods adopted for the determination of chlorpheniramine in pharmaceutical formulations were found in the literature including, polarography [170], spectrophotometry [171, 172], infra red spectroscopy [173], micellar liquid chromatography [174, 175], capillary electrophoresis [176, 177], HPLC and capillary electrophoresis [178], ion-selective sensors [179], HPLC [180- 183], HPLC and spectroscopy [184] and atomic emission spectrophotometry [185]. Most of the methods mentioned above have some drawbacks. Generally, they are lengthy procedures and consume a lot of reagents and time. It is therefore necessary to find a more effective, economical, automated and specific method as an alternative.

2.7.3 The proposed SIA method

An automated and cost-effective SIA method was proposed for the assay of chlorpheniramine maleate in pharmaceutical formulations. The method was based on the oxidation of chlorpheniramine by potassium permanganate in sulphuric acid media. The highly controlled conditions of the method made the spectrophotometric measurements easier to be performed. This method was accompanied by a complete chemometric optimization of the variables to help perform a comprehensive procedure for the drug.

2.7.4 SIA program controlling chlorpheniramine assay

A suitable SIA manifold for the oxidation of chlorpheniramine was constructed (figure 2.8), and the appropriate SIA protocol to execute the procedure was programmed (Appendix 7). The points below describe the consequent steps followed to execute the SIA method.

1. Water was linked to the syringe pump through in-position mode to push reagents to the required part of the SIA manifold.

2. Sulphuric acid, permanganate, chlorpheniramine and water were linked to the selector valve through ports 2, 3, 4 and 5 respectively.
3. The syringe was filled with 1500 µl of water by directing the bi-directional valve to the in-position mode with flow rate of 150 µl s^{-1}.
4. The Teflon tubes were filled with their respective reagents by performing aspirations runs and directing the bi-directional valve to the out-position mode.
5. With the same fast flow rate, the syringe was emptied and step 3 was repeated.
6. 35 µl of sulphuric acid, 30 µl of permanganate and 50 µl of water were sequentially aspirated into the holding coil and short reverse strokes were performed three times to allow the mixing of the reagents to take place at flow rate 25 µl s^{-1}.
7. With a flow rate of 50 µl s^{-1}, 250 µl volumes was dispensed to the reaction coil and the mixture was delayed for the required time to allow the reaction to take place.
8. 1200 µl volume was dispensed with a flow rate of 25 µl s^{-1} to the Z-flow cell passing through the reaction coil and at the same time to the reference and absorbance scan were carried out at wavelength 507, 526, and 546 nm, and the maximum absorbance was recorded as the value a_1.
9. The syringe is emptied and refilled with 1500 µl of water.
10. To ensure the repeatability of dispersion and mixing, steps 6, 7, 8 and 9 were repeated three times and the average of a_1 (last two measurements) was calculated as A_1.
11. 35 µl of sulphuric acid, 30 µl of permanganate, 30 µl of chlorpheniramine and 20 µl of water were sequentially aspirated into the holding coil and short reverse strokes were performed three times to allow the mixing of the reagents at flow rate 25 µl s^{-1}.
12. With a flow rate of 50 µl s^{-1}, 250 µl volumes was dispensed to the reaction coil and the mixture was delayed for the required time to allow the reaction to take place.

13. 1200 µl volume was dispensed with a flow rate of 25 µl s^{-1} to the Z-flow cell passing through the reaction coil and at the same time to the reference and absorbance scan were carried out at wavelength 507, 526, and 546 nm and the maximum absorbance was recorded as the value a_2.
14. The syringe is emptied and refilled with 1500 µl of water.
15. To ensure the repeatability of monitoring the reaction, steps 11, 12, 13 and 14 were repeated three times and the average of a_2 (last two measurements) was calculated as A_2.

    The response R of the reaction was calculated using equation 2.2.

$$R = A_1 - A_2 \qquad (2.2)$$

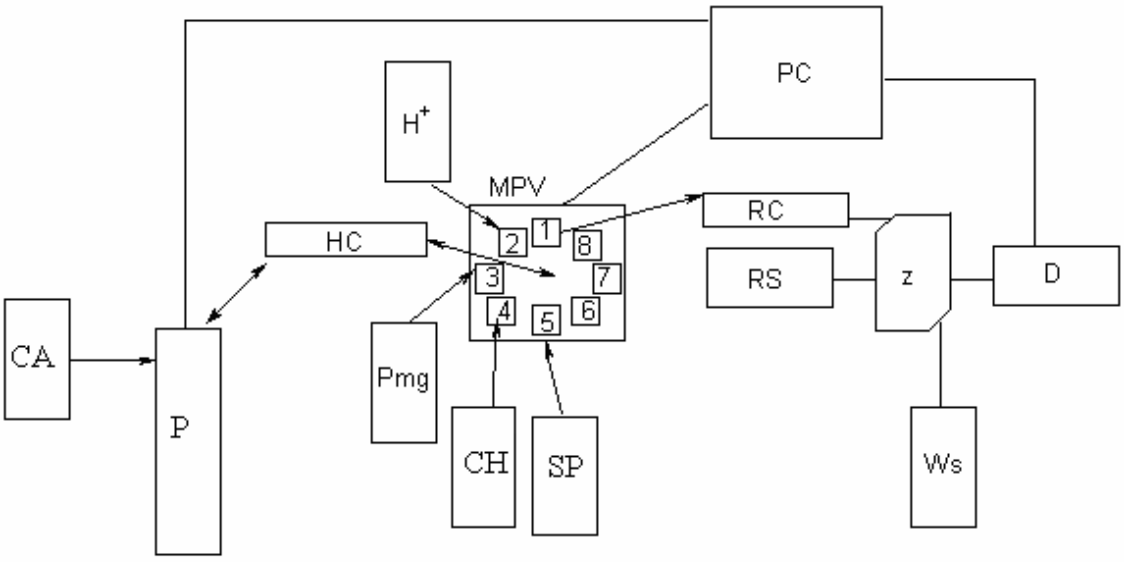


Fig. 2.8: Sequential injection analyzer manifold diagram (CH), CA: carrier (water); P: syringe pump; HC: holding coil; H^+: sulphuric acid; Pmg: potassium permanganate; CH: Chlorpheniramine analyte; MPV: multi-position valve; 1-8: ports; RC: reaction coil; SP: spacer (water) RS: radiation source; Z: Z-flow cell; D: detector; Ws: waste; PC: personal computer.

2.8 Verapamil hydrochloride Assay

2.8.1 Introduction

Verapamil hydrochloride is chemically named as 2-(3, 4-dimethoxyphenyl)-5-[{2-(3, 4-dimethoxyphenyl) ethyl} (methyl) amino]-2-(1-methylethyl)pentanenitrile hydrochloride (figure 2.9). It is a white, crystalline powder, soluble in water, sparingly soluble in alcohol and freely soluble in methanol [132]

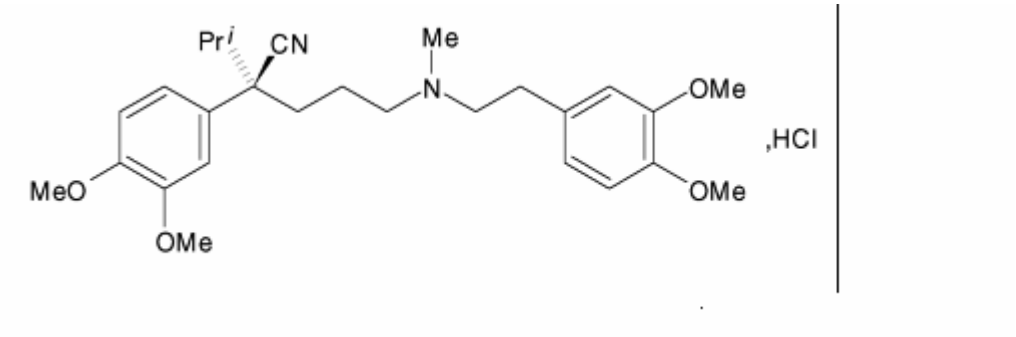


Figure 2.9.: Verapamil hydrochloride structure

Verapamil is a calcium-channel blocker and a class IV antiarrythmic drug. Verapamil slows conduction through the atrioventricular node, and thus slows the increased ventricular response rate that occurs in atrial fibrillation and flutter. A decrease in both coronary and peripheral vascular resistance together with a sparing effect on myocardial intracellular oxygen consumption appears to be the modes of action in angina. The decreased peripheral vascular resistance may explain the antihypertensive effect of verapamil. It is used in the control of supraventricular arrhythmias and in the management of angina pectoris and hypertension. It may also be used in the management of myocardial infarction.
Verapamil hydrochloride is usually available as mono component in dosage forms containing injections and tablets [169].

2.8.2 Assay methods of verapamil hydrochloride in pharmaceutical formulations

Many analytical methods for the assay of verapamil hydrochloride in pharmaceutical preparations were established. Several methods were proposed

involving official standard methods as well as non-official standard methods. The sections below describe the advantages and disadvantages of some used methods.

2.8.2.1 Official standard methods

The BP [132] method for the assay of verapamil hydrochloride in the generic form is a potentiometric titration with sodium hydroxide after dissolving the sample in ethanol and hydrochloric acid. A direct spectrophotometry is provided for the injection form; the absorbance is measured at wavelength 278 nm after dilution with a 0.01 mol l^{-1} hydrochloric acid. The tablet form is assayed by shaking verapamil with 0.1mol l^{-1} hydrochloric acid for 10 minutes and then adding sufficient 0.1 mol l^{-1} hydrochloric acid and dilution of the filterate with water. The absorbance is measured at wavelength 278nm. This method is lengthier compared with that of the injection form.

2.8.2.2 Other non-official methods

Some analytical methods adopted for the determination of verapamil in pharmaceutical formulations were reported in the literature including, spectrophotometry [186], HPLC [187], atomic emission spectrophotometry [188], adsorptive stripping voltammetry [189], flow injection and pulse amperometric [190], micellar liquid chromatography [191], ion-selective electrode [192], and Resonance Rayleigh scattering with flow injection [193]. Most of these methods have some limitations. They are rather lengthy and consume a lot of reagents and time. So, it is advantageous to find a more effective, economical, automated and specific method.

2.8.3 The proposed SIA method

An automated and cost-effective SIA method was proposed for the assay of verapamil hydrochloride in pharmaceutical formulations. The method was based on the oxidation of verapamil by potassium permanganate in sulphuric acid media. The highly controlled conditions of the method made the

spectrophotometric measurements much easier to be performed. This method was accompanied by a complete chemometric optimization of the variables to help perform a comprehensive assay procedure for proper determination of the drug under study.

2.8.4 SIA program controlling verapamil hydrochloride assay

A suitable SIA manifold for the oxidation of verapamil hydrochloride was constructed (figure 2.10), and the appropriate SIA protocol to execute the procedure was programmed (Appendix 6). The points below describe the consequent steps followed to execute the SIA method.

1. Water was linked to the syringe pump through in-position mode to push reagents to the required part of the SIA manifold.
2. Sulphuric acid, permanganate, verapamil and water were linked to the selector valve through ports 2, 3, 4 and 5 respectively.
3. The syringe was filled with 1500 µl of water by directing the bi-directional valve to the in-position mode with flow rate of 150 µl s^{-1}.
4. The Teflon tubes were filled with their respective reagents by performing aspirations runs and directing the bi-directional valve to the out-position mode.
5. With the same fast flow rate, the syringe was emptied and step 3 was repeated.
6. 35 µl of sulphuric acid, 30 µl of permanganate and 50 µl of water were sequentially aspirated into the holding coil and short reverse strokes were performed three times to allow the mixing of the reagents to take place at flow rate 25 µl s^{-1}.
7. With a flow rate of 50 µl s^{-1}, 250 µl volumes was dispensed to the reaction coil and the mixture was delayed for the required time to allow the reaction to take place.
8. 1200 µl volume was dispensed with a flow rate of 25 µl s^{-1} to the Z-flow cell passing through the reaction coil and at the same time to the reference

and absorbance scan were carried out at wavelength 507, 526, and 546 nm, and the maximum absorbance was recorded as the value a.

9. The syringe is emptied and refilled with 1500 µl of water.
10. To ensure the repeatability of dispersion and mixing, steps 6, 7, 8 and 9 were repeated three times and the average of the last two measurements was calculated as A.
11. 35 µl of sulphuric acid, 30 µl of permanganate, 30 µl of verapamil and 20 µl of water were sequentially aspirated into the holding coil and short reverse strokes were performed three times to allow the mixing of the reagents at flow rate 25 µl s^{-1}.
12. With a flow rate of 50 µl s^{-1}, 250 µl volumes was dispensed to the reaction coil and the mixture was delayed for the required time to allow the reaction to take place.
13. 1200 µl volume was dispensed with a flow rate of 25 µl s^{-1} to the Z-flow cell passing through the reaction coil and at the same time to the reference and absorbance scan were carried out at wavelength 507, 526, and 546 nm, and the maximum absorbance was recorded as the value b.
14. The syringe is emptied and refilled with 1500 µl of water.
15. To ensure the repeatability of monitoring the reaction, steps 11, 12, 13 and 14 were repeated three times and the average of b (last two measurements) was calculated as B

    The response R of the reaction was calculated using equation 2.3.

$$R = A - B \qquad (2.3)$$

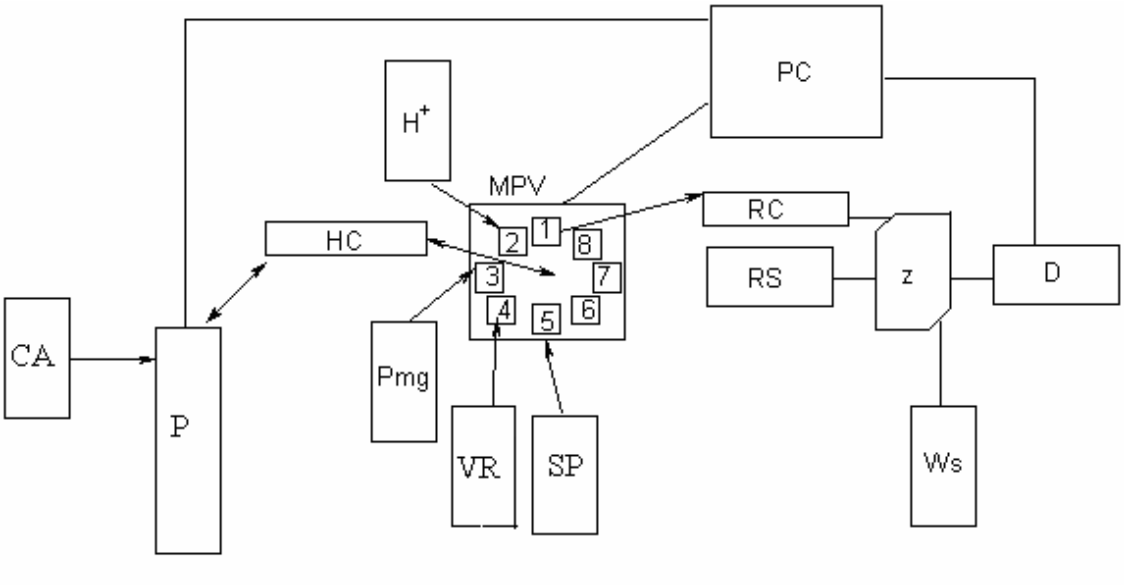


Fig. 2.10: Sequential injection analyzer manifold diagram (VR), CA: carrier (water); P: syringe pump; HC: holding coil; H^+: sulphuric acid; Pmg: potassium permanganate; VR: Verapamil analyte; MPV: multi-position valve; 1-8: ports; RC: reaction coil; SP: spacer (water) RS: radiation source; Z: Z-flow cell; D: detector; Ws: waste; PC: personal computer.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Chlorpheniramine maleate assay

The utilization of SIA for the assay of chlorpheniramine in pharmaceutical formulations adopted spectrophotometric method of detection that is, by measuring the absorbance difference as explained by equation 2.2. In the following sections the results obtained are discussed and justified.

3.1.1 Optimization of variables

Key parameters that influence the performance of the proposed SIA method were studied in order to establish the optimum working conditions. Both physical and chemical parameters were studied.

3.1.1.1 Physical parameters

Physical parameters of the SIA system control the extent of the reaction and the degree of dispersion of a product of a reaction between the analyte and the colored reagent before it reaches the detector. This is important because the response is directly proportional to the concentration of the product passing through the flow cell. These parameters are responsible for delivering appreciable amount of the product for detection.

3.1.1.1.1 Wavelength determination

In order to obtain results with minimum interferences, it will be better to identify the wavelength which is optimum for giving reasonable peak height. The proposed system relies mainly on the measurements of these peak heights. In permanganate spectrum wavelengths of 507nm, 526nm and 546nm respectively were found to show some absorbance. The wavelength of 546nm gave the best results and was the one to be used.

3.1.1.1.2 Time

The reaction between chlorpheniramine and permanganate in acidic medium was based on the oxidation of chlorpheniramine by permanganate and consequent measurement of the absorbance differences between the blank which

is permanganate alone and after adding the analyte as explained by equation 2.2. Since this reaction is not fast enough, therefore the time must be optimized. A chemometric optimization for time was done as shown in figures 3.3, 3.4, 3.5 and 3.6, and 4 minutes time was found to be the best.

3.1.1.1.3 Flow rate

The flow rate should be optimized so that the contact period is sufficient. The best flow rate was found to be 50µl s^{-1} for the carrier solution and 25µl s^{-1} from the multiposition valve to the holding coil on the reverse direction towards the detector and through the reaction coil.

3.1.1.1.4 Volume

The volumes of the reagents and analyte used throughout the method were kept constant; 30µl chlorpheniramine, 30µl permanganate; 35µl acid and 20µl of spacer solution.

3.1.1.1.5 Effect of HC and RC dimensions

The dimensions of the holding coil have a limited effect once this coil can accommodate the volumes without diffusion into the carrier stream. The reaction coil dimensions have an influence on the dispersion degree and the extent of the reaction whether it was fast or slow because the zones are propelled through it and towards the detector and the length of 160 cm was used.

3.1.1.2 Chemical parameters

The proposed SIA system for the determination of chlorpheniramine is based on the reaction with potassium permanganate in acidic medium at wavelength 546 nm. The concentrations of each of these chemicals determine the limit of detection and the linear range of this system that can be used for quantitative analysis

3.1.1.2.1 Effect of sulphuric acid concentration

Preliminary studies showed that sulphuric acid gave the best results; these results are evaluated and it was found that the optimum concentration ranges between 1×10^{-3}mol l^{-1} and 1×10^{-2}mol l^{-1}. The higher concentration gave better results, but it seems to interfere somehow in the reaction, that the peak heights tend to get shorter as the time passes, which were supposed to be of the same height. The concentration of 1×10^{-3}mol l^{-1} was used. A complete chemometric optimization of the effect of acid was done as shown in figures 3.1, 3.2, 3.3 and 3.4.

3.1.1.2.2 Effect of permanganate concentration

Permanganate seems to have a profound effect on the process and as it has strong oxidizing capabilities and high molar absorptivity, it was used with success in this system with concentrations ranging from 5×10^{-4} to 5×10^{-3}mol l^{-1}. The reproducible and best results were found to be between 5×10^{-4} and 1×10^{-3} mol l^{-1}. A concentration of 1×10^{-3}mol l^{-1} was used. A complete chemometric optimization of the effect of permanganate was performed as shown in figures 3.1, 3.2, 3.5 and 3.6.

3.1.2 Sequential chemometric optimization

Sequential chemometric optimization was employed to find out the optimum operating conditions of the proposed system and to check the parameters which substantially affected the efficiency of the method.

3.1.2.1 The Experimental Design

The experimental design approach was employed and 2^k factorial design was run where 2 stands for variable levels considering the higher and lower values and k is the number of factors studied. Three factors were studied including sulphuric acid and permanganate concentrations and the delay time. The highest and lowest values were determined and assigned +1 and -1 coded levels respectively, and the matrix was arrayed as shown in table 3.1. The lowest

and the highest values were determined based on the same criteria discussed above. Eight experiments as a result of a 2^3 factorial design were arranged and the original levels and their responses were introduced as shown in table 3.2.

3.1.2.1.1 The response surface method

The results obtained from the factorial design were interpolated and plotted using SigmaPlot® software package. The surface plot of the response as a function of sulphuric acid concentration and permanganate concentration levels were plotted as in figure 3.1and 3.2. It was obvious that the surface response increases as the concentration of permanganate increases and to some extent as the concentration of acid increases. The figures indicate that the effect of permanganate concentration on the response was more than that of the acid concentration. The surface plot as a function of sulphuric acid concentration and delay time is presented in figures 3.3. It shows that at low acid concentration there is slight effect on response, but high acid concentration the response increases as time decreases. Figure 3.4 shows that at high and low acid concentrations response increases as time increases, but the effect on the response was higher at high acid concentration. Figure 3.5 and figure 3.6 show the surface plot of the response as a function of permanganate concentration and delay time. The effect of permanganate concentration on the response increases as the delay time increases.

Table3.1:
Full treatment combinations of the coded values of parameters controlling the assay method of chlorpheniramine

Exp. Number	H_2SO_4	$KMnO_4$	Time	Response
1	+1	+1	-1	0.1960
2	+1	+1	+1	0.2285
3	-1	+1	-1	0.1705
4	-1	+1	+1	0.1895
5	-1	-1	-1	0.1045
6	-1	-1	+1	0.1010
7	+1	-1	-1	0.1230
8	+1	-1	+1	0.1145

Table3.2:
Response obtained with the full treatment combinations of the original values of parameters controlling the assay method of chlorpheniramine

Exp. Number.	H_2SO_4	$KMnO_4$	Time	Response
1	0.01	0.001	2 min	0.1960
2	0.01	0.001	4 min	0.2285
3	0.001	0.001	2 min	0.1705
4	0.001	0.001	4 min	0.1895
5	0.001	0.0005	2 min	0.1045
6	0.001	0.0005	4 min	0.1010
7	0.01	0.0005	2 min	0.1230
8	0.01	0.0005	4 min	0..1145

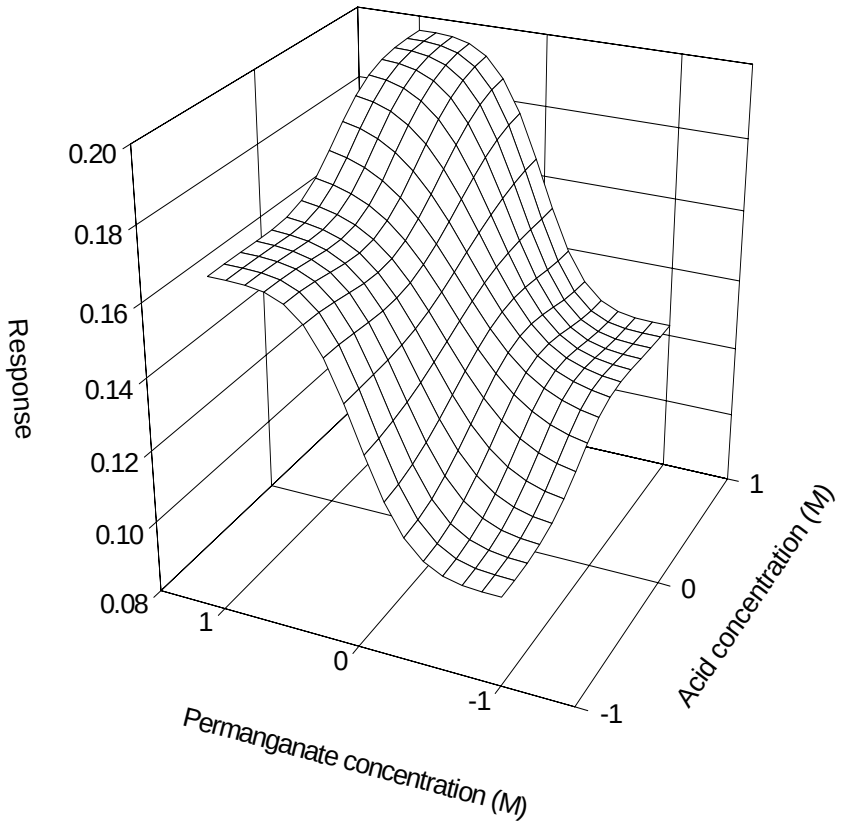


Fig. 3.1: Response surface plot of acid concentration (mol l^{-1}) against permanganate concentration (mol l^{-1}) for chlorpheniramine (time 2 min)

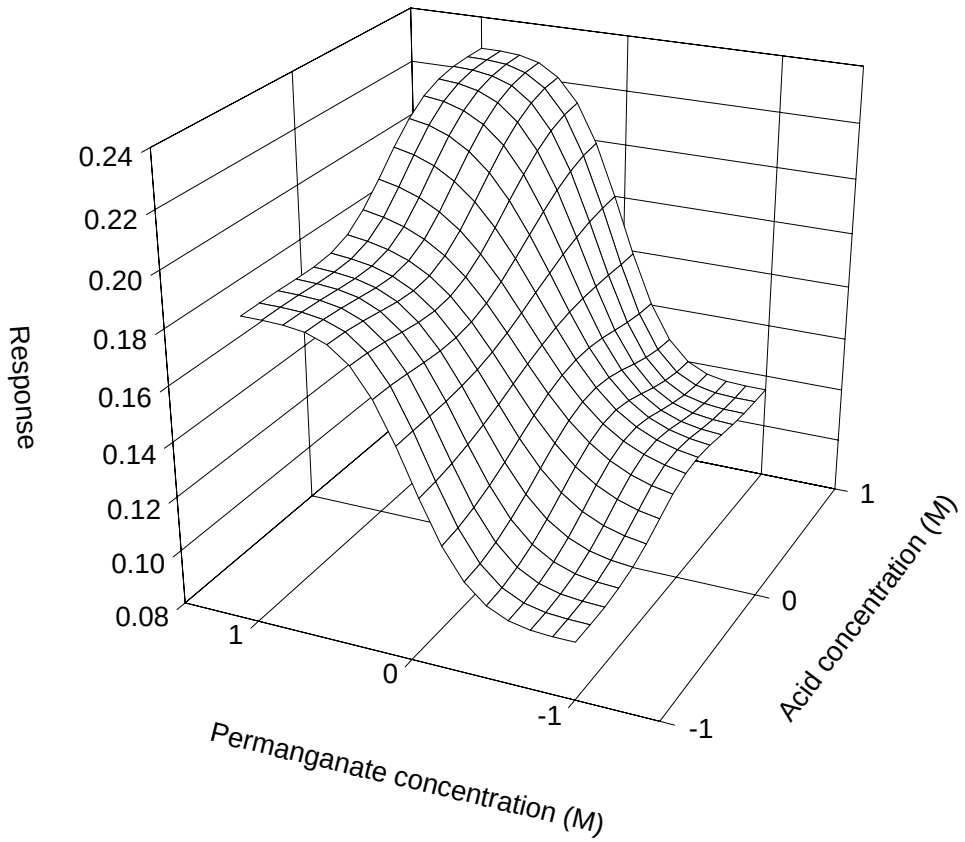


Fig. 3.2: Response surface plot of acid concentration (mol l^{-1}) against permanganate concentration (mol l^{-1}) for chlorpheniramine (time 4 min)

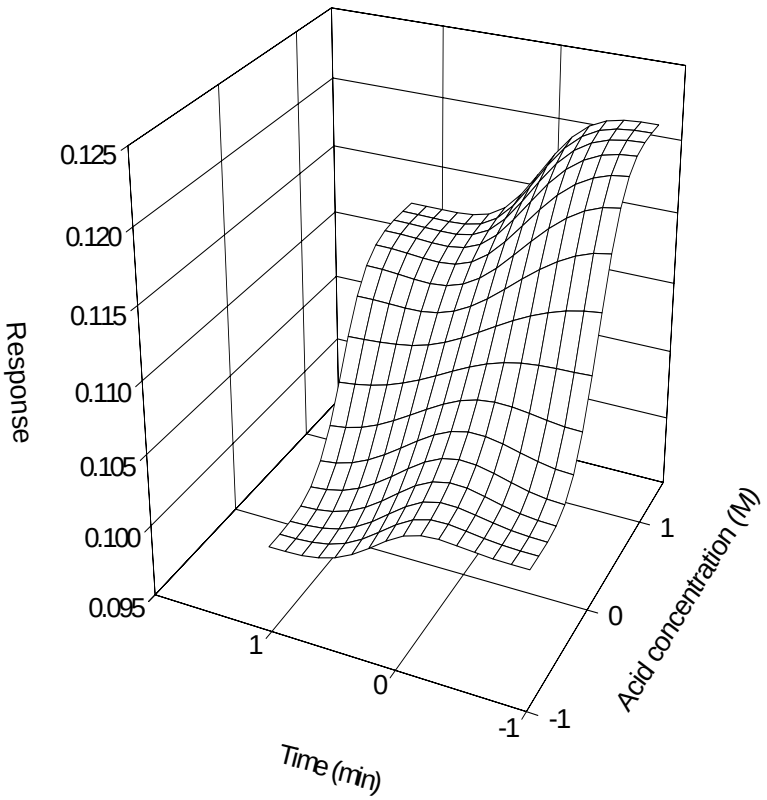


Fig. 3.3: Response surface plot of acid concentration (mol l^{-1}) against time for chlorpheniramine (permanganate 5×10^{-4} mol l^{-1})

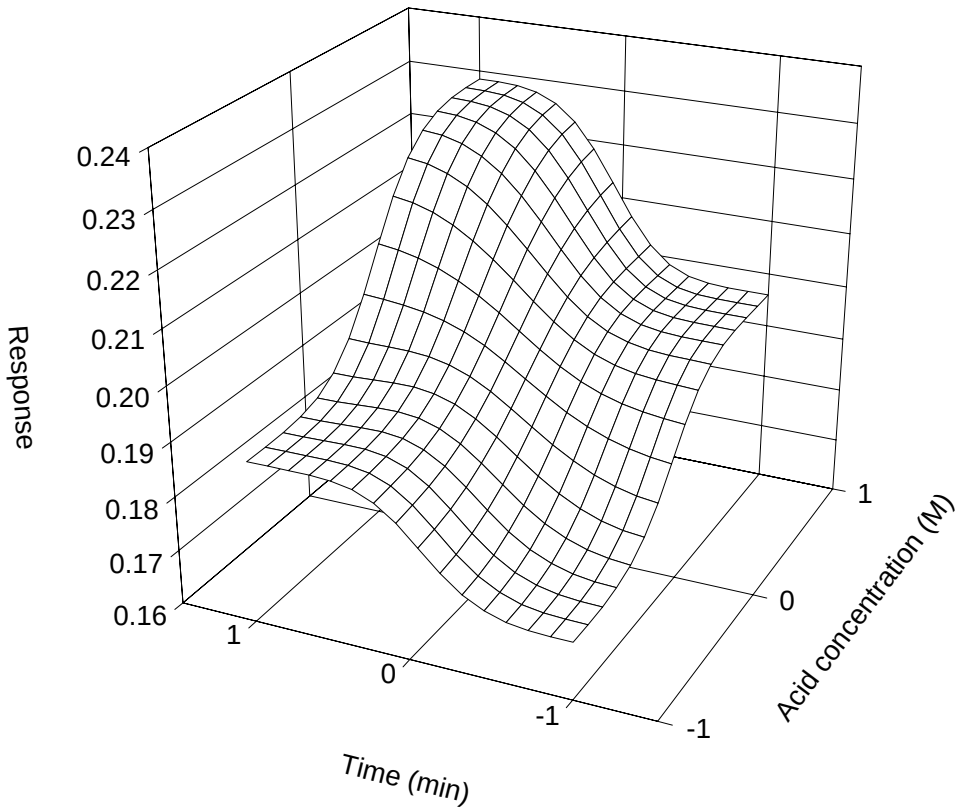


Fig. 3.4: Response surface plot of acid concentration (mol l^{-1}) against time for chlorpheniramine (permanganate 1×10^{-3} mol l^{-1})

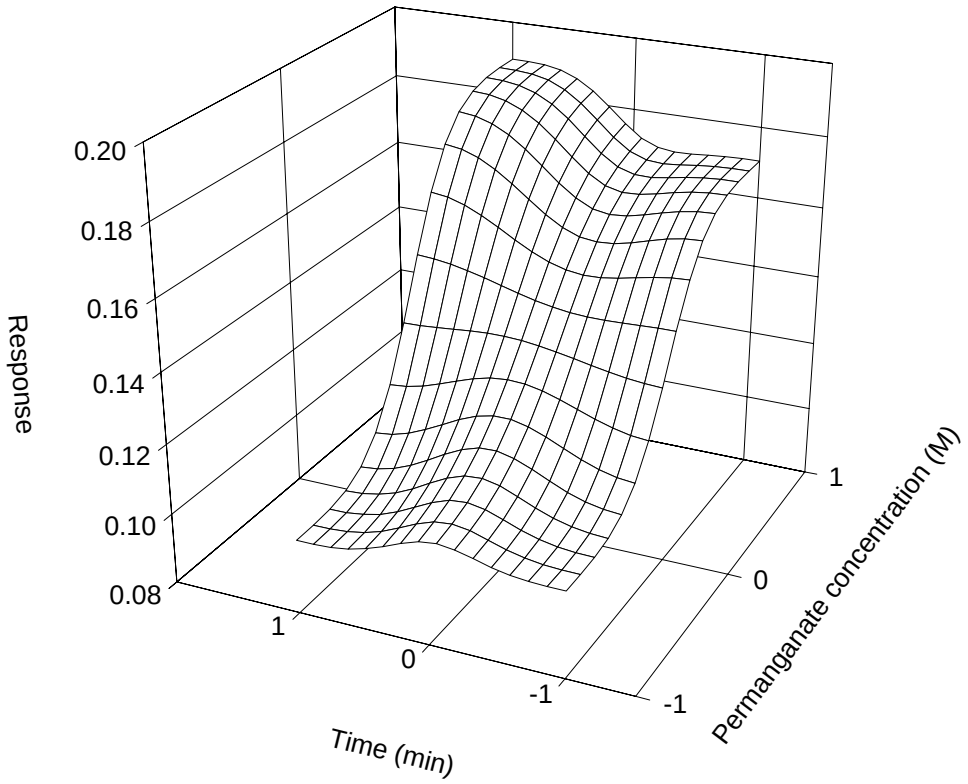


Fig. 3.5: Response surface plot of permanganate concentration (mol l^{-1}) against time for chlorpheniramine (acid 1×10^{-3} mol l^{-1})

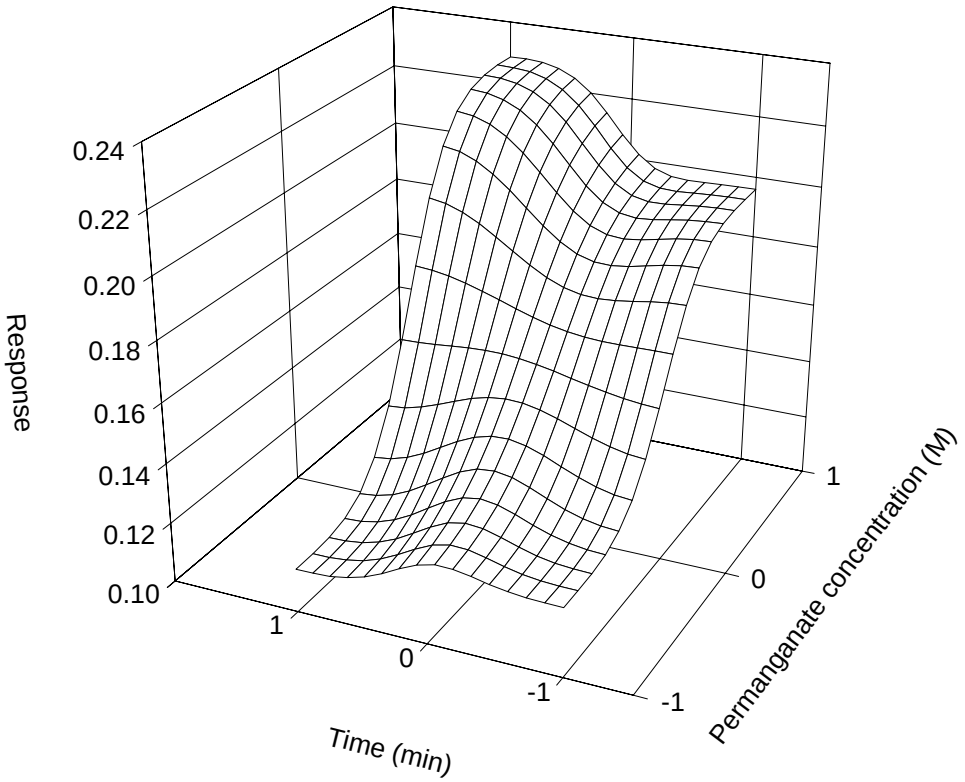


Fig. 3.6: Response surface plot of permanganate concentration (mol l^{-1}) against time for chlorpheniramine (acid 1×10^{-2} mol l^{-1})

3.1.2.1.2 The Estimation of Effects

Another method of the ANOVA approach was applied i.e. the effect factor (E_f) on the response. The main factor of a variable explains the level of effect on the response of a system. It is calculated as the difference between the average of the response of the highest levels (+1) and the average of the response of the lowest levels (-1) as presented in Eq. 3.1. Therefore a 2^3 factorial design was adopted to calculate the effect factors. The interaction effect factor explains the level of the interaction effect between variables on the response of a system. In this respect, the encoded levels of each experiment are multiplied and then calculated as in Eq. 3.1. Table 3.3 shows a 2^3 factorial design matrix and the multiplication of the encoded levels of the variables. The main and interaction effect factors were calculated and the results obtained were introduced in table 3.4. The main effect factors show that permanganate concentration had a significant effect on the response more than the effect of acid concentration or delay time. The two-variable interaction effect factor between permanganate concentration and delay time was higher than that between acid concentration and permanganate concentration or time. The three-variable interactions effect was lower than permanganate concentration and delay time, acid and permanganate concentrations and higher than that of acid and delay time interaction effect. These findings strengthen what was explained by the surface plot that permanganate concentration and delay time strongly interacted with each other than with acid concentration.

$$E_f = \frac{\sum Y(+1)}{n} - \frac{\sum Y(-1)}{n} \qquad 3.1$$

Table 3.3
A 2^3 factorial design matrix and variables interaction

Exp. No.	A	P	T	AP	AT	PT	APT	Response
1	+1	+1	-1	+	-	-	-	0.1960
2	+1	+1	+1	+	+	+	+	0.2285
3	-1	+1	-1	-	+	-	+	0.1705
4	-1	+1	+1	-	-	+	-	0.1895
5	-1	-1	-1	+	+	+	-	0.1045
6	-1	-1	+1	+	-	-	+	0.1010
7	+1	-1	-1	-	-	+	+	0.1230
8	+1	-1	+1	-	+	-	-	0.1145

A: acid concentration, P: permanganate concentration, T: delay time,
R: response

Table 3.4
The effect of variables on the response

Effect factor	**Variable**	**Value**
Main effect	A	0.0242
	P	0.0853
	T	0.0098
Two-variable interaction	AP	0.0081
	AT	0.0021
	PT	0.0158
Three-variable interaction	APT	0.0046

3.1.3 Analytical appraisals

The developed method was validated in order to evaluate if adequate linearity, repeatability, recovery, precision and accuracy had been achieved. The linearity of the proposed SIA system for the determination of chlorpheniramine maleate was evaluated under the optimum conditions. A series of standard solutions of chlorpheniramine were prepared and applied. Figure 3.7 shows the calibration plot of chlorpheniramine by the four standard solutions with the concentrations of 20, 50, 100 and 150 ppm. Beer's law was obeyed for this concentration range. The regression calibration equation obtained under optimum conditions was

$$R = 0.0016C + 0.0064 \qquad 3.2$$

Where R is the response calculated by the equation 2.1 and C is the unknown concentration of chlorpheniramine maleate as ppm. The correlation coefficient r was found to be 0.9998 indicating good linearity.

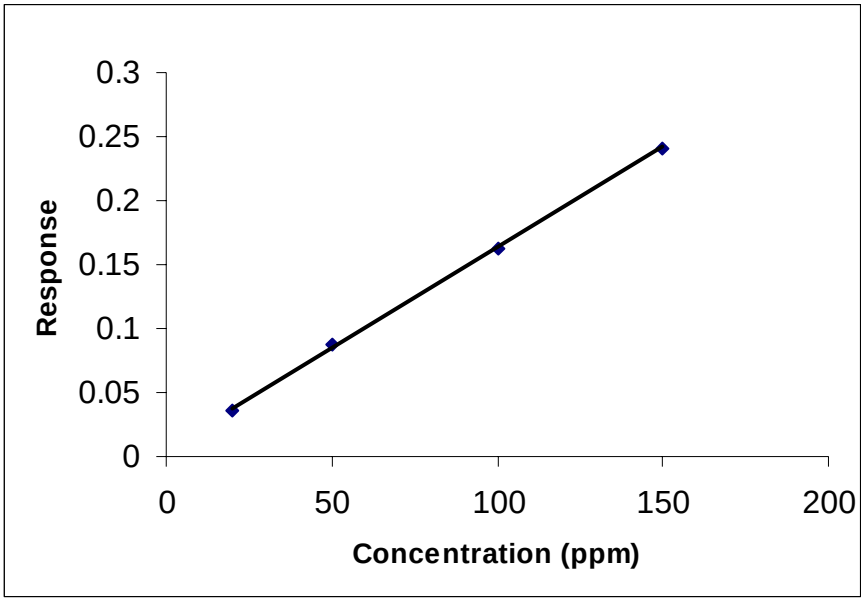


Figure 3.7 SIA calibration plot of Chlorpheniramine
(1×10^{-3} mol l^{-1}) M H_2SO_4, (1×10^{-3} mol l^{-1}) $KMnO_4$, 4min)

3.1.4 Application

The optimized SIA method was applied for the assay of chlorpheniramine maleate in pharmaceutical preparations collected from drug stores. Starch as one of the expected additive was examined to make sure that it did not to interfere with the process of determination of the analyte. Blank measurements using only the oxidizing agent which is permanganate and after adding 10 ppm starch were conducted and the absorbance difference was calculated as shown in equation 2.1. This revealed that the excipient added showed negligible interference with the whole process. The mean recovery and the RSD% (relative standard deviation) are presented in table 3.5. The results obtained showed acceptable accuracy and repeatability.

Table 3.5 Results obtained by the SIA and BP methods for the analysis of chlorpheniramine maleate synthetic sample, tablets and injections

Chlorpheniramine sample	SIA	BP	Official range*	Recovery %	R. S. D. ♠ %
Synthetic sample	99.8%	98%	98-101%	101.8%	± 1.8%
Allerfin 10mg injection	96%	95%	90-110%	101%	± 1%
Anallerg 4 mg tablets	97.25%	98.3%	92.5-107.5%	98.9%	± 1.1%

*The official range of the content of a drug specified by the BP.
♠The R. S. D. of n =7

3.2 Verapamil hydrochloride Assay

3.2.1 Optimization of variables

Key parameters that influence the performance of the proposed SIA method were studied in order to establish the optimum working conditions. Both physical and chemical parameters were studied

3.2.1.1 Physical parameters

Physical parameters of the SIA system control the extent of the reaction and the degree of dispersion of a product of a reaction between the analyte and the colored reagent before it reaches the detector. This is important because the response is directly proportional to the concentration of the product passing through the flow cell. These parameters are responsible for delivering appreciable amount of the product for detection.

3.2.1.1.1 Wavelength determination

In order to obtain results with minimum interferences, it will be best to identify the optimum wavelength that for giving reasonable peak height, since the proposed system relies mainly on the measurements of these peak heights. In permanganate spectrum wavelengths of 507nm, 526nm and 546nm respectively were found to show some absorbance. The wavelength of 546nm gave the best results and it was the one to chosen.

3.2.1.1.2 Time

The reaction between verapamil and permanganate in acidic medium is based on the oxidation of verapamil by permanganate and consequent measurement of the absorbance differences between the blank which is permanganate alone and after the addition of the analyte as explained by equation 2.3. For this reaction the time must be optimized. A chemometric optimization for time was done as shown in figures 3.10, 3.11, 3.12 and 3.13 and 2 minutes time was the best to be used since delay time of 4 minutes did not improve the peak heights and were almost the same as for that of 2 minutes.

3.2.1.1.3 Flow rate

The flow rate should be optimized so that the contact period is sufficient and the best flow rate was found to be 50µl s^{-1} for the carrier solution and 25µl $s^{-1}$from the multiposition valve to the holding coil on the reverse direction towards the detector and through the reaction coil.

3.2.1.1.4 Volume

The volume of the reagents and the analyte used throughout the method was kept constant; 30µl verapamil, 30µl permanganate; 35µl acid and 20µl of spacer solution which was water.

3.2.1.1.5 Effect of HC and RC dimensions

The dimensions of the holding coil have a limited effect once this coil can accommodate the volumes without diffusion into the carrier stream. The reaction coil dimensions have an influence on the dispersion degree and the extent of the reaction whether it was fast or slow because the zones are propelled through it and towards the detector and its length was 160 cm.

3.2.1.2 Chemical parameters

The proposed SIA system for the determination of verapamil is based on the reaction with potassium permanganate in acidic medium at wavelength 546nm.The concentrations of each of these chemicals determine the limit of detection and the linear range of this system that can be used for quantitative analysis

3.2.1.2.1 Effect of sulphuric acid concentration:

Preliminary studies showed that sulphuric acid gave the best results. These results are evaluated and it was found that the optimum concentration ranges between 1×10^{-3}mol l^{-1} and 1×10^{-2}mol l^{-1}. The higher concentration gave better results, but it seems to interfere somehow in the reaction so that the peak heights tend to get shorter as the time passes, though supposed to be of the same

height. The concentration of 1×10^{-3}mol l^{-1} was selected. A complete chemometric optimization of the effect of acid was done as shown in the figures, 3.8, 3.9, 3.10 and 3.11.

3.2.1.2.2 Effect of permanganate concentration:

Permanganate seems to have a profound effect on the process since it has enjoying strong oxidizing capabilities and high molar absorptivity; it is used with success in this system with concentrations ranging from 1×10^{-3} to 3×10^{-3}mol l^{-1}. The reproducible and best results were found to be between 1×10^{-3} and 2×10^{-3} mol l^{-1}. A concentration of 2×10^{-3}mol l^{-1} was the one used in this assay method. A complete chemometric optimization of the effect of permanganate was performed as shown in figure 3.8, 3.9, 3.12 and 3.13.

3.2.2 Sequential chemometric optimization

Sequential chemometric optimization was employed to find out the optimum operating conditions of the proposed system and to check the parameters which substantially affected the efficiency of the method.

3.2.2.1 Experimental design

Experimental design is a powerful tool when used in part of optimization. Ruggedness testing and modeling of chemical analysis procedures were usually employed to understand the relationship between experimental factors and their corresponding responses, as well as the determination of the optimum working conditions at which minor changes of these factors can be tolerated. Experimental design approach along with response surface modeling were employed and 2^k factorial design was run where 2 stands for variable levels considering the higher and lower values and k is the number of factors studied. Three factors were studied including sulphuric acid and permanganate concentrations and the delay time. The highest and lowest values were determined and assigned +1 and -1 coded levels respectively, and the matrix was arrayed as shown in table 3.6. The lowest and the highest values were

determined based on the same criteria discussed above. Eight experiments as a result of a 2^3 factorial design were arranged and the original levels and their respective responses were introduced as shown in table 3.7.

3.2.2.1.1 The surface response method

The results obtained from the factorial design were interpolated and graphed using SigmaPlot® software package. The surface plot of the response as a function of sulphuric acid concentration and permanganate concentration levels were plotted as in figure 3.8 and 3.9. In figure 3.8 it was obvious that the surface response increases as the concentration of permanganate increases and to some extent as the concentration of acid increases. Figure 3.9 shows that at high acid concentration there is slight increase in the response, but at low acid concentration there is a significant increase in response. The figures indicate that the effect of permanganate concentration on the response was more than that of the acid concentration. The surface plot as a function of sulphuric acid concentration and delay time is presented in figures 3.10 and 3.11. In figure 3.10 there is slight increase of response with respect to acid concentration and delay time. Figure 3.11at high acid concentration and time did not show any effect while at low acid concentration the response increases with the increase of delay time. Figure 3.12 and figure 3.13 show the surface plot of the response as a function of permanganate concentration and delay time. In figure 3.12 the effect of delay time on the response is higher than that of permanganate concentration.

Table3.6
Full treatment combinations of the coded values of parameters controlling the assay method of verapamil hydrochloride

Exp. Number	H_2SO_4	$KMnO_4$	Time	Response
1	-1	-1	-1	0.1009
2	-1	-1	+1	0.1175
3	-1	+1	-1	0.1755
4	-1	+1	+1	0.2160
5	+1	-1	-1	0.1455
6	+1	-1	+1	0.1620
7	+1	+1	-1	0.2435
8	+1	+1	+1	0.2400

Table3.7
Response obtained with the full treatment combinations of the original values of parameters controlling the assay method of verapamil hydrochloride

Exp. Number.	H_2SO_4	$KMnO_4$	Time	Response
1	0.001	0.001	2 min	0.1009
2	0.001	0.001	4 min	0.1175
3	0.001	0.002	2 min	0.1755
4	0.001	0.002	4 min	0.2160
5	0.01	0.001	2 min	0.1455
6	0.01	0.001	4 min	0.1620
7	0.01	0.002	2 min	0.2435
8	0.01	0.002	4 min	0.2400

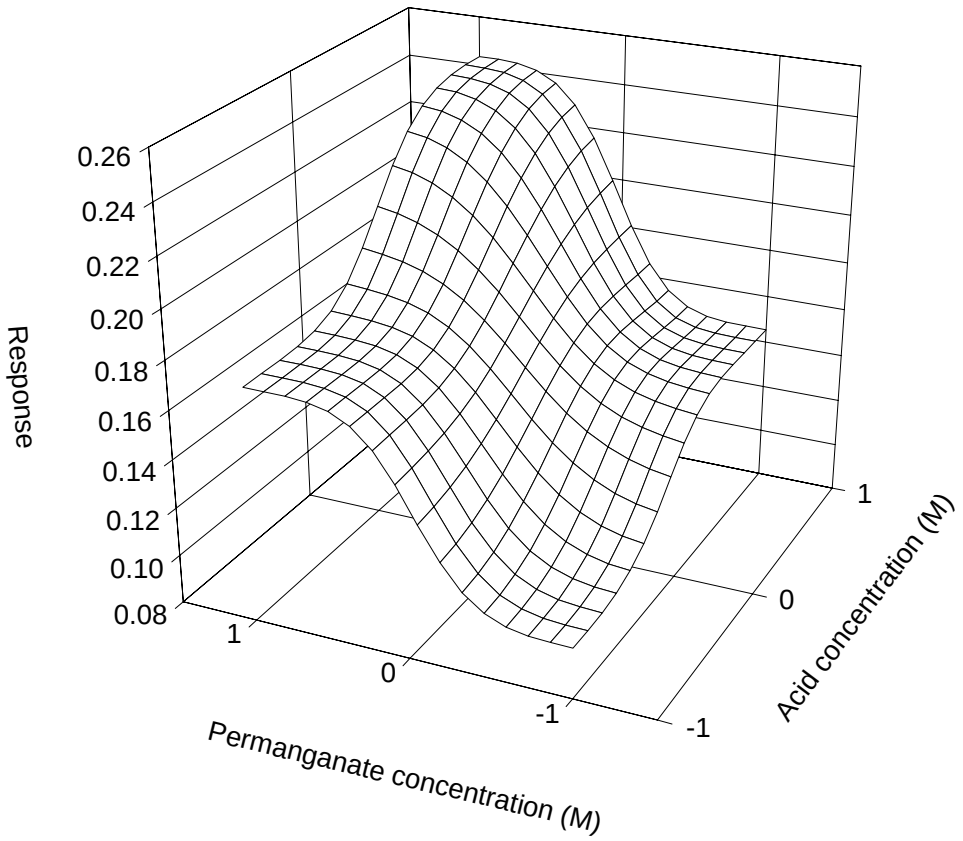


Fig. 3.8: Response surface plot of acid concentration (mol l^{-1}) against permanganate concentration (mol l^{-1}) for verapamil (time 2 min)

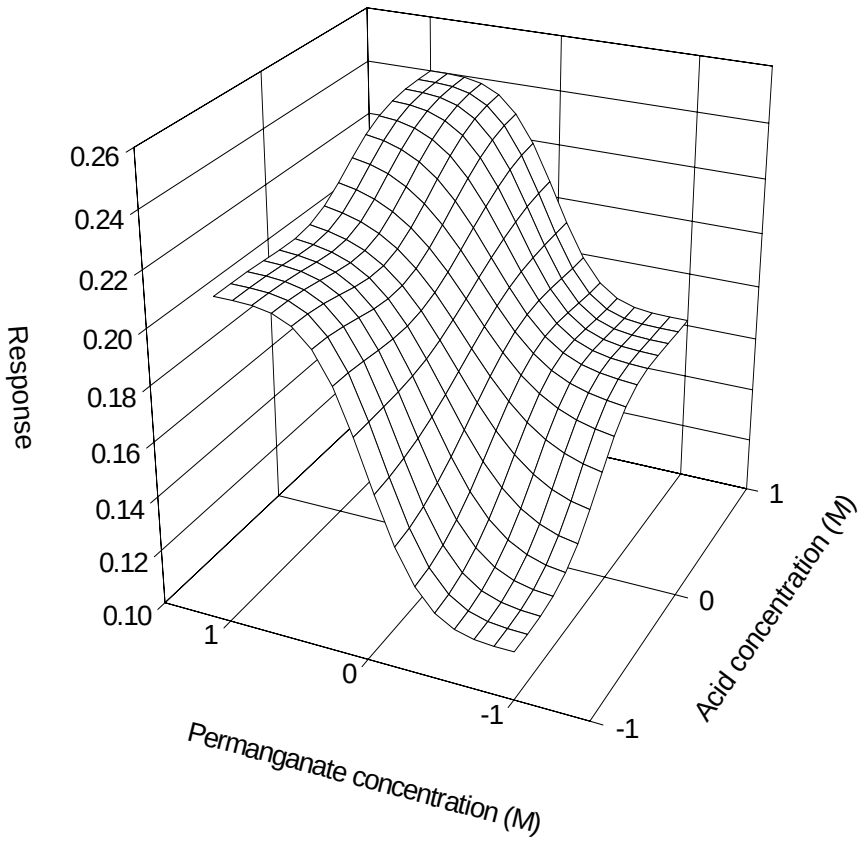


Fig. 3.9: Response surface plot of acid concentration (mol l^{-1}) against permanganate concentration (mol l^{-1}) for verapamil (time = 4 min)

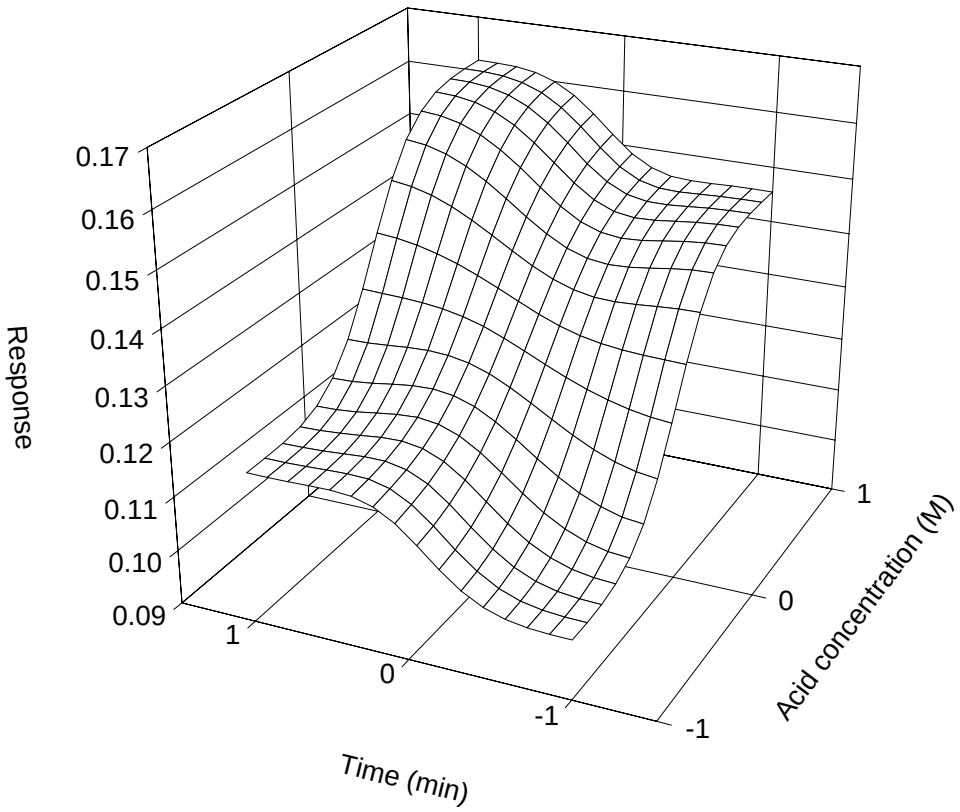


Fig.3.10: Response surface plot of acid concentration (mol l^{-1}) against time for verapamil (permanganate 1×10^{-3} mol l^{-1})

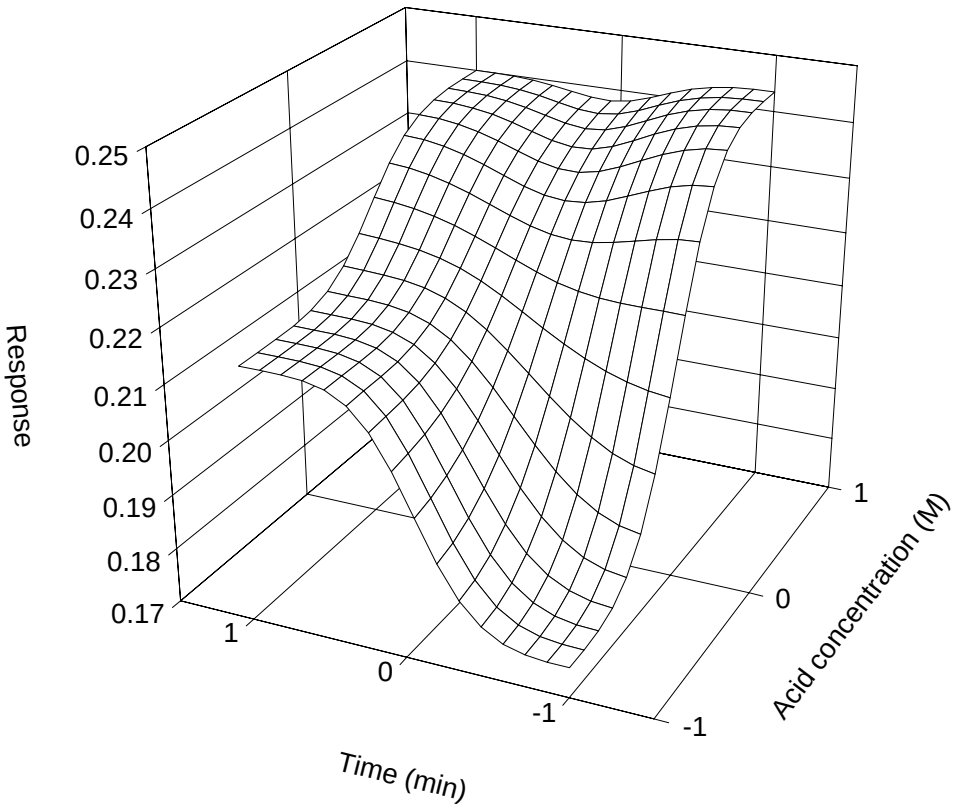


Fig. 3.11: Response surface plot of acid concentration (mol l^{-1}) against time for verapamil (permanganate 2×10^{-3} mol l^{-1})

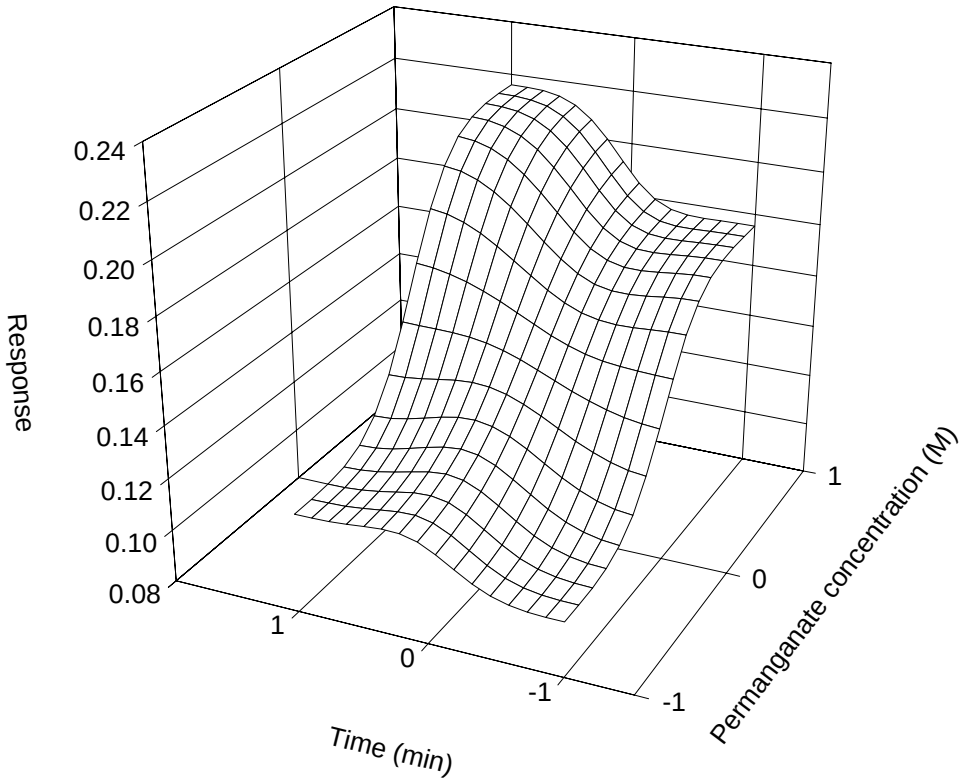


Figure.3.12: Response surface plot of permanganate concentration (mol l^{-1}) against time for verapamil (acid 1×10^{-3} mol l^{-1})

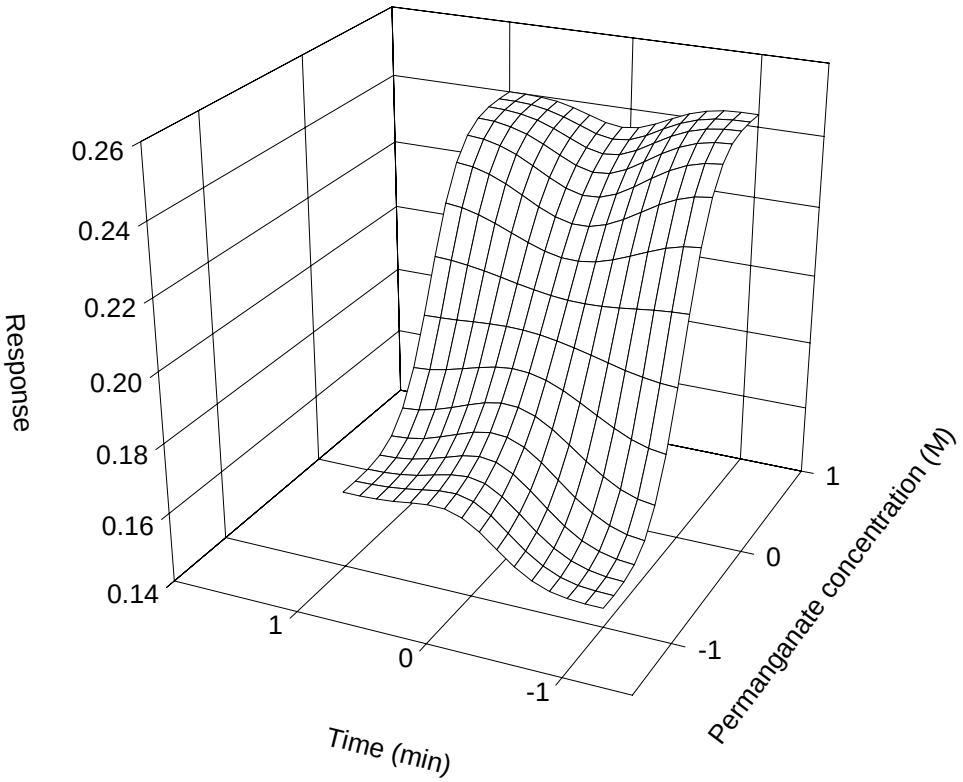


Figure: 3.13: Response surface plot of permanganate concentration (mol l^{-1}) against time for verapamil (acid 1×10^{-2} mol l^{-1})

3.2.2.1.2 Estimation of Effects

Another method of the ANOVA approach was applied i.e. the effect factor (E_f) on the response. The main factor of a variable explains the level of effect on the response of a system. It is calculated as the difference between the average of the response of the highest levels (+1) and the average of the response of the lowest levels (-1) as presented in Eq. 3.1. Therefore a 2^3 factorial design was adopted to calculate the effect factors. The interaction effect factor explains the level of the interaction effect between variables on the response of a system. In this respect, the encoded levels of each experiment are multiplied and then calculated as in Eq. 3.1. Table 3.8 shows a 2^3 factorial design matrix and the multiplication of the encoded levels of the variables. The main and interaction effect factors were calculated and the results obtained were introduced in table 3.9. The main effect factors show that permanganate concentration had a more significant effect on the response more than the effect of acid concentration or delay time respectively. The two-variable interaction effect factor between acid concentration and time was higher than that between acid and permanganate concentrations and delay time, permanganate concentration and time and acid and permanganate respectively. However, the effect factor study exhibits positive effect for all factors except for acid time and acid permanganate time which have negative effect. These findings strengthen what was explained by the surface plot that permanganate concentration and delay time strongly interacted with each other than with acid concentration.

Table 3.8
A 2^3 factorial design matrix and variables interaction

Exp. No.	A	P	T	AP	AT	PT	APT	Response
1	-1	-1	-1	+	+	+	-	0.1009
2	-1	-1	+1	+	-	-	+	0.1175
3	-1	+1	-1	-	+	-	+	0.1755
4	-1	+1	+1	-	-	+	-	0.2160
5	+1	-1	-1	-	-	+	+	0.1455
6	+1	-1	+1	-	+	-	-	0.1620
7	+1	+1	-1	+	-	-	-	0.2435
8	+1	+1	+1	+	+	+	+	0.2400

A: acid concentration, P: permanganate concentration, T: delay time,
R: response

Table 3.9
The effect of variables on the response

Effect factor	**Variable**	**Value**
Main effect	A	0.0452
	P	0.0873
	T	0.0173
Two-variable interaction	AP	0.00072
	AT	-0.011
	PT	0.00098
Three-variable interaction	APT	-0.0109

3.2.3 Analytical appraisals

The developed method was validated in order to evaluate if adequate linearity, repeatability, recovery, precision and accuracy had been achieved. The linearity of the proposed SIA system for the determination of verapamil hydrochloride was evaluated under the optimum conditions. A series of standard solutions of the analyte were prepared and applied. Figure 3.10 shows the calibration plot of verapamil by the four standard solutions with the concentrations of 50, 100, 150 and 200 ppm. Beer's law was obeyed for this concentration range. The regression calibration equation obtained under optimum conditions was

$$R = 0.0018C + 0.005 \qquad 3.3$$

Where R is the response calculated by equation 2.1 and C is the unknown concentration of verapamil hydrochloride as ppm. The correlation coefficient r was found to be 0.9978 indicating good linearity.

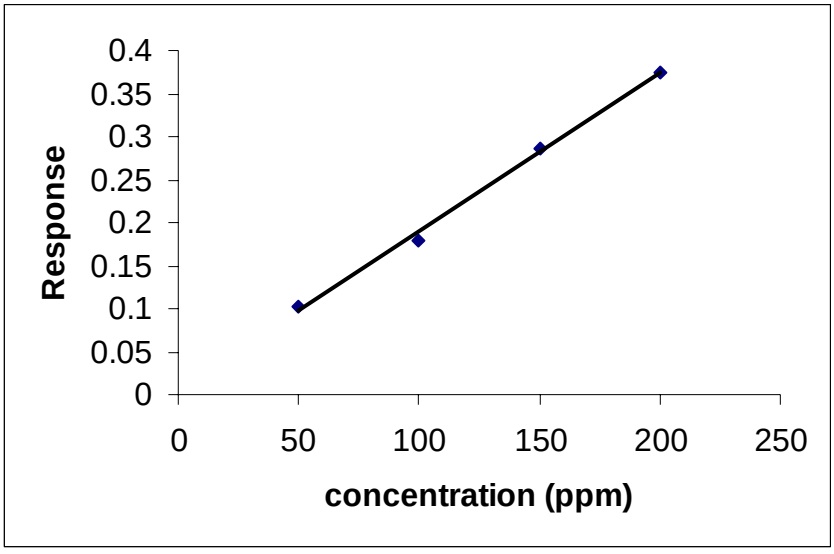


Figure 3.10: SIA calibration plot of Verapamil
(1×10^{-3}mol l^{-1} H_2SO_4, 2×10^{-3}mol l^{-1} $KMnO_4$, 2min)

3.2.4 Application

The accuracy of the proposed SIA method was evaluated by comparing the results obtained for samples with the proposed SIA method with those performed with BP standard method. The % R.S. D. (relative standard deviation) and the mean recovery of verapamil hydrochloride are presented in table 3.10. The results from both methods were found to be comparable and show acceptable accuracy and repeatability. Verapamil hydrochloride is available in pharmaceutical formulations as a mono drug, so only excipients such as starch, talc or maltose could be present in a typical pharmaceutical formulation. A blank measurement was run with permanganate alone and after adding 10 ppm starch to examine the interference of excipients. The absorbance of the blank was almost the same as that after adding starch.

Table 3.10 Results obtained by the SIA and BP methods for the analysis of verapamil hydrochloride synthetic sample, tablets and injections

Verapamil sample	SIA	BP	Official* range	recovery %	R. S. D. ♠ %
Synthetic sample	99.8%	101%	99-101%	98.81	±1.2
Manidon 5 mg injection	101%	102%	90-110%	99.01	±0.98
Isoptin 40 mg tablets	98.89	96%	92.5-107.5%	103	±3.01

*The official range of the content of a drug specified by the BP.
♠The R. S. D. n =7

3.3 Promethazine hydrochloride Assay

Promethazine hydrochloride assay was based on its oxidation by cerium (IV) in sulfuric acid medium. Many factors may affect this process including supporting electrolye concentration, current, flow rate and type of electrodes. The potential of polarized electrode will be changed and will be different from that at zero current. Anodic polarization will make the potential more positive than zero current, while cathodic polarization results in negative potential. This difference in potential is due to overpotential.

For oxidation-reduction (redox) reactions, the indictor electrode is usually a noble metal of the platinum group, gold or silver under limited conditions. This electrode will serve to transfer electrons from the solution to the external circuit without being oxidized itself. Optimization of factors that affect electrolysis will lead to a good detection.

3.3.1 Optimization of variables

Key parameters that influence the performance of the proposed DEP method were studied in order to establish the optimum working conditions.

3.3.1.1 Flow rate

The flow rate should be optimized so that the contact period is sufficient and the best flow rate was found to be 20µl s^{-1} for the addition of the reagent into the glass cell.

3.3.1.2 DC Current

The current needed to polarize the electrodes was optimized and the best current to be used was 5µA which was kept constant throughout the analysis.

3.3.1.3 Type of electrodes

Two identical platinum electrodes were used. They were fixed in specific position in the glass cell and immersed in the solution in a way not to touch the bottom of the cell to avoid acquiring any kind of resistance. The electrodes were

connected to electronic circuit and ammeter, and then the differential electrolytic potential between the two polarized electrodes was measured by the voltmeter.

3.3.1.4.1Effect of sulphuric acid concentration

Preliminary studies showed that sulphuric acid as supporting electrolyte gave the best results as it facilitates transfer of electrons during electrolysis. Adding supporting electrolyte in a proper concentration is sufficient to lower the resistance potential to an insignificant value. Rapid stirring of solution results in a uniform concentration in the bulk. Since ions carry the current, electromigration of reacting species will occur. The addition of a background electrolyte minimizes such migration which depends upon the conductivity of the electrolyte solution and the amount of current flowing but not on electrochemical processes. These results are evaluated and it was found that the optimum concentration ranges between 1×10^{-3}mol l^{-1} and 1×10^{-2}mol l^{-1}.

3.3.1.5 Effect of Cerium (IV) concentration:

Cerium (IV) in sulfuric acid seems to have a profound effect on the process and since it has strong oxidizing capabilities, it is used with great success in this system with concentrations ranging from 5×10^{-5} to 1×10^{-3}mol l^{-1} with respect to different concentrations of the analyte. The procedure was based on the oxidization of promethazine hydrochloride by cerium (IV) in acidic medium and generation of a potential difference between the two indicator electrodes in the electrolyte solution which is detected electrically by a voltmeter connected to the system as shown in figure 2.6.

3.3.2 DEP Titration curves

Unlike normal potentiometric titrations in which the endpoint is determined by plotting the potential against the volume or the ratio of $\Delta E/\Delta V$ against the volume as in figure 3.15, in DEP (differential electrolytic potentiometric) titration curves the endpoint will be determined directly at the highest point of the curve of differential potentiometric potential against volume.

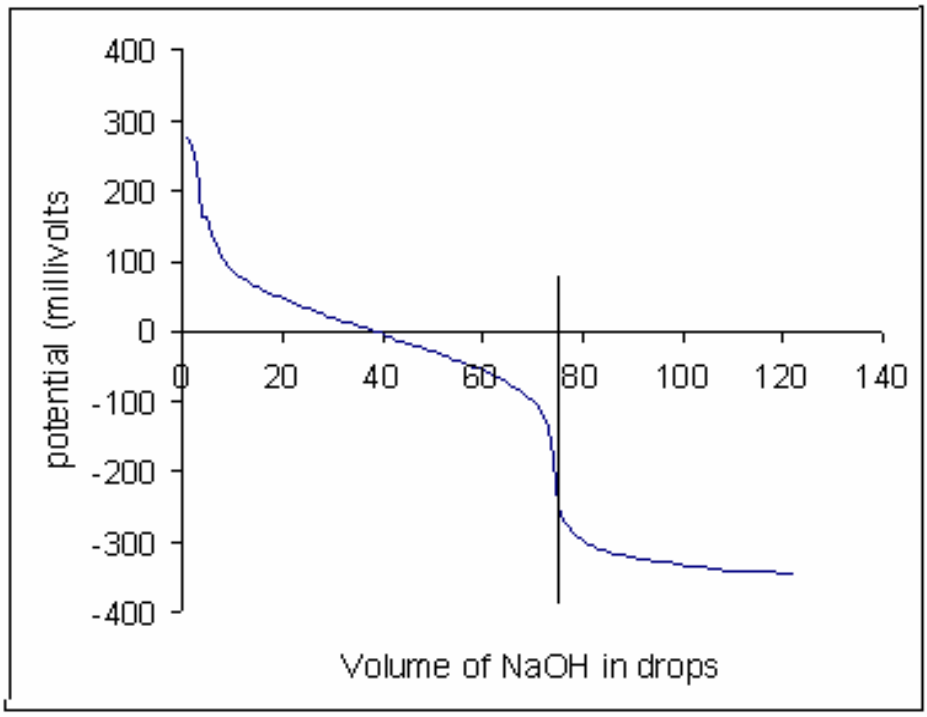


Fig. 3.15: A typical potentiometric titration curve of promethazine hydrochloride against NaOH

3.3.2.1 Determination of the end point

To determine the end point for each titration, a DEP titration curve was graphed by plotting the volume of cerium (IV) in microliters against the DE potential in millivolts, the highest point of the curve corresponding to the highest potential reading will be the endpoint and the volume corresponding to this potential will be read from the graph (figure 3.16.) Usually we are concerned with the volume near the end point.

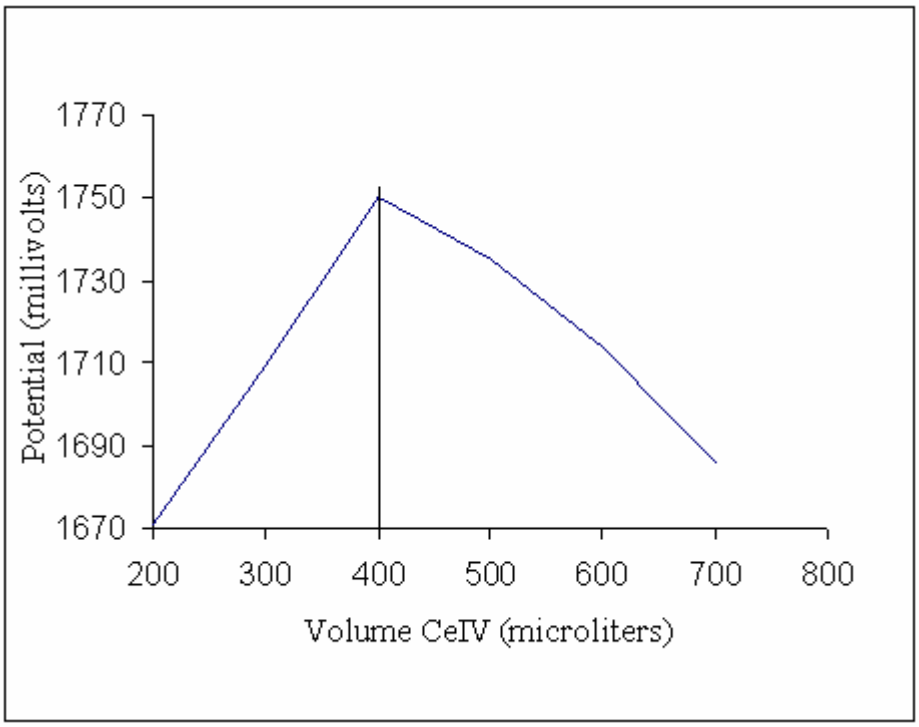


Fig.3.16: DEP titration curve of 1 ml of 5×10^{-4}mol l^{-1} promethazine with 1×10^{-3}mol $l^{-1}$of Ce (IV) and 2ml of 5×10^{-3}mol l^{-1} H_2SO_4

3.3.2.2 Determination of Cerium (IV) volume

The determination of Cerium (IV) volume will lead in turn to the determination of the number of moles of cerium (IV) that reacted with promethazine hydrochloride. To do this a number of DEP titrations were performed under different conditions. The differential electrolytic potential was recorded and graphed against the volume of cerium (IV).

3.3.2.3 Analytical calculations

To calculate the number of moles of cerium (IV) that reacted with promethazine, first the volume reading was taken from the graph, and then the rule in equation 3.4 was applied.

$$\text{Number of moles} = \frac{\text{molarity x volume (ml)}}{1000} \qquad 3.4$$

To calculate the number of moles for Ce (IV) and for promethazine hydrochloride reacted with each other.

(1) In figure 3.17:

For Ce (IV), 8×10^{-4}mol l^{-1} × 0.7(ml)/1000 = 5.6 x 10^{-7} moles

For promethazine HCl, 5×10^{-4}mol l^{-1} x 1(ml)/1000 =5×10^{-7}moles

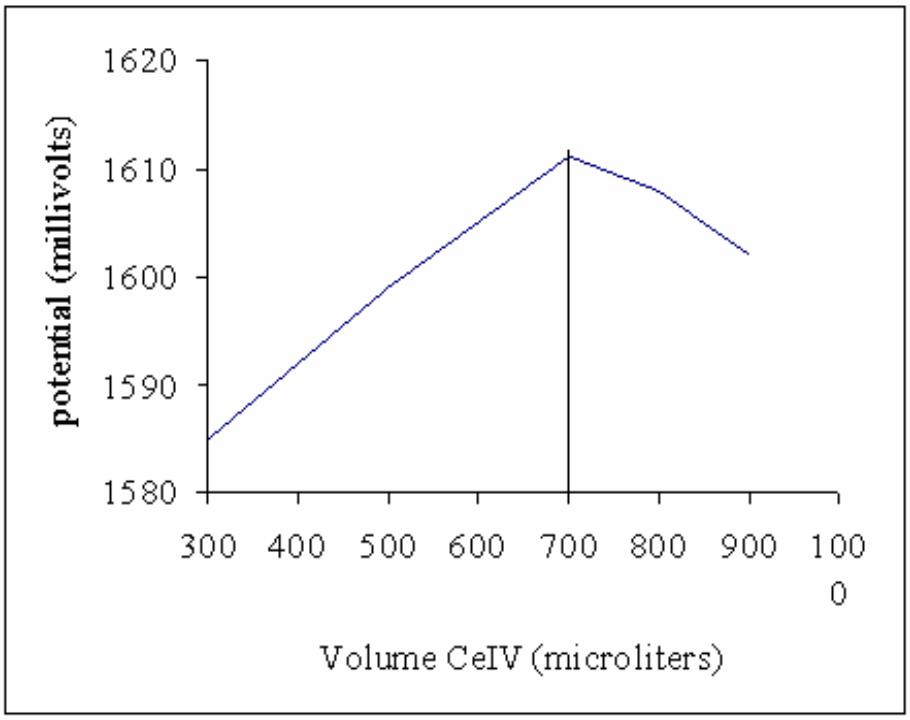


Fig.3.17: DEP titration curve of 1 ml of 5×10^{-4}mol l^{-1} promethazine with 8×10^{-4}mol $l^{-1}$of Ce (IV) and 2ml of 5×10^{-3}mol l^{-1} H_2SO_4

(2) In figure 3.18:

For Cerium (IV), 4×10^{-4}mol l^{-1} × 0.9(ml)/1000 = 3.6 x 10^{-7} moles

For promethazine HCl, 5×10^{-4}mol l^{-1} x 1(ml)/1000 =5×10^{-7}moles

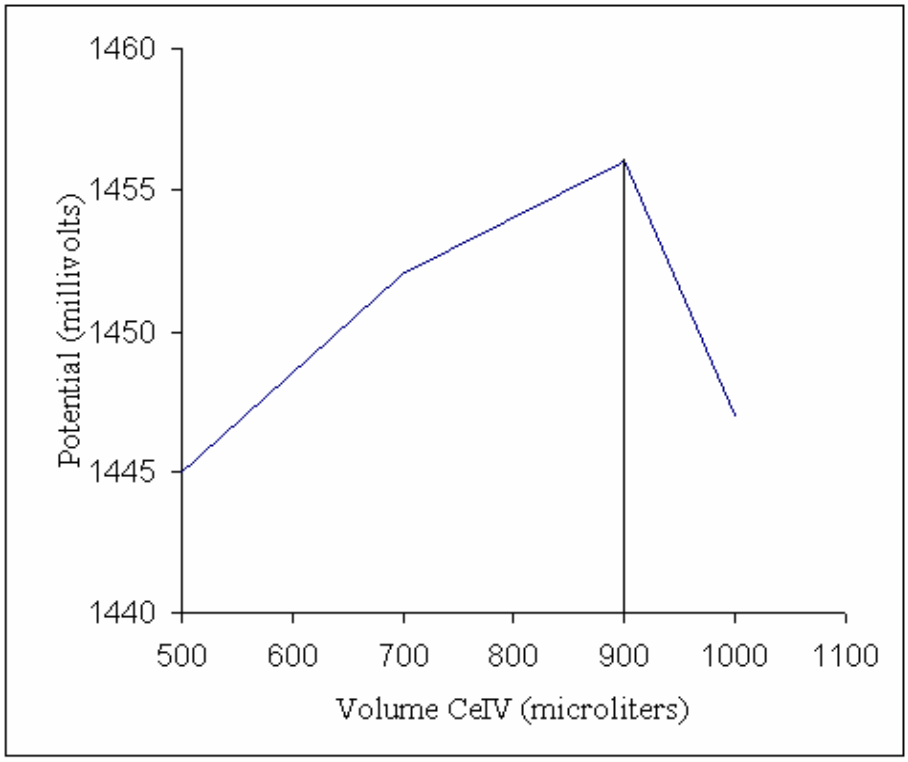


Fig.3.18: DEP titration curve of 1 ml of 5×10^{-4}mol l^{-1} promethazine with 4×10^{-4}mol $l^{-1}$of Ce (IV) and 2ml of 5×10^{-3}mol l^{-1} H_2SO_4

(3) In figure 3.19:

For Cerium (IV), 2.5×10^{-4}mol l^{-1} × 0.5(ml)/1000 = 1.25 x 10^{-7} moles

For promethazine HCl, 1.25×10^{-4}mol l^{-1} x 1(ml)/1000 =1.25×10^{-7}moles

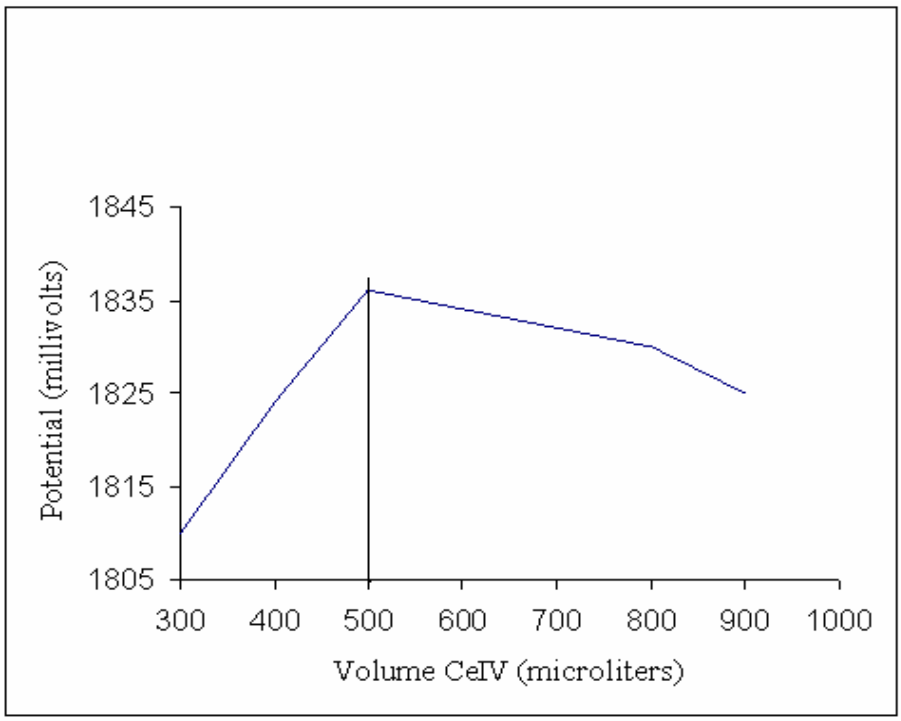


Fig.3.19: DEP titration curve of 1 ml of 1.25×10^{-4}mol l^{-1} promethazine with 2.5×10^{-4}mol $l^{-1}$of Ce (IV) and 2ml of 1×10^{-3}mol l^{-1} H_2SO_4

(4) In figure 3.20:

For Cerium (IV), 2.5×10^{-4}mol l^{-1} × 0.5(ml)/1000 = 1.25 x 10^{-7} moles

For promethazine HCl, 1.25×10^{-4}mol l^{-1} x 1(ml)/1000 =1.25×10^{-7}moles

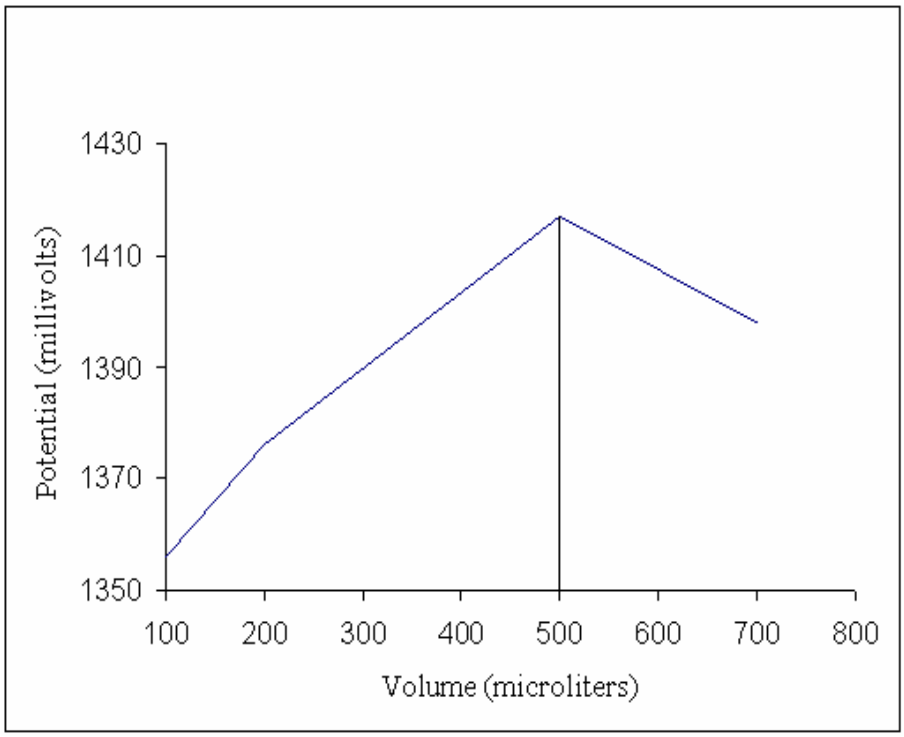


Fig.3.20: DEP titration curve of 1 ml of 1.25×10^{-4}mol l^{-1} promethazine with 2.5×10^{-4}mol $l^{-1}$of Ce (IV) and 2ml of 5×10^{-3}mol l^{-1} H_2SO_4

(5) In figure 3.21:

For Cerium (IV), 2.5×10^{-4}mol l^{-1} × 0.6(ml)/1000 = 1.5 x 10^{-7} moles

For promethazine HCl, 2.5×10^{-4}mol l^{-1} x 0.5(ml)/1000 =1.25×10^{-7}moles

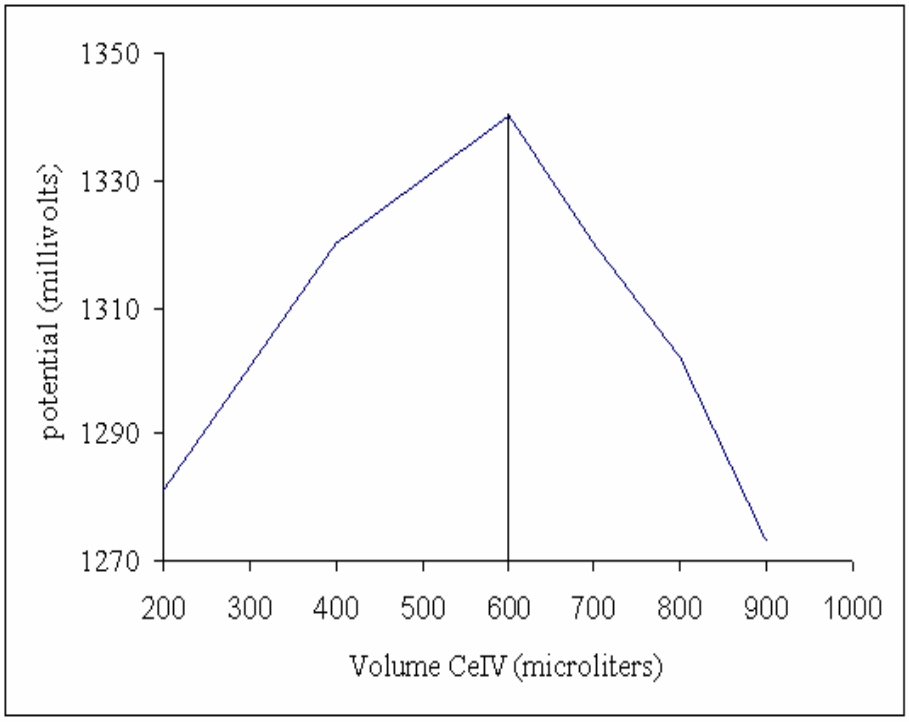


Fig.3.21: DEP titration curve of 0.5 ml of 2.5×10^{-4}mol l^{-1} promethazine with 2.5×10^{-4}mol $l^{-1}$of Ce (IV) and 2ml of 1×10^{-3}mol l^{-1} H_2SO_4

(6) In figure 3.22:

For Cerium (IV), 5×10^{-5}mol l^{-1} × 0.8(ml)/1000 = 4 x 10^{-8} moles

For promethazine HCl, 3×10^{-5}mol l^{-1} x 1(ml)/1000 =3×10^{-8}moles

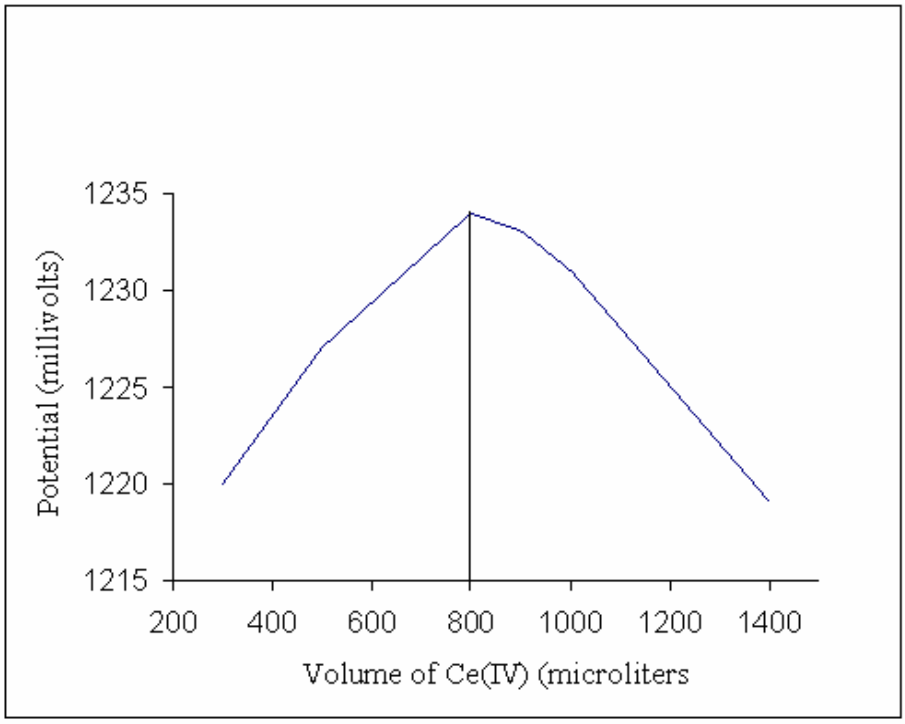


Figure 3.22: DEP titration curve of 1 ml of 3×10^{-5}mol l^{-1} promethazine with 5×10^{-5}mol $l^{-1}$of Ce (IV) and 2ml of 1×10^{-3}mol l^{-1} H_2SO_4

3.3.2.4 Impression

Promethazine hydrochloride was found to be oxidizable by cerium (IV) in sulfuric acid media resulting in a free radical form [161] and it was shown in (figure 3.23). In this study and from the above findings it was obvious that one mole of cerium (IV) reacted with one mole of promethazine hydrochloride.

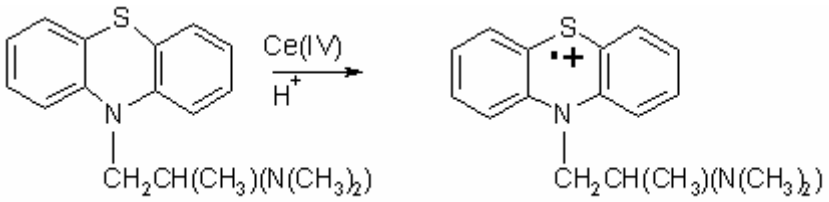


Fig.2.23: The proposed reaction scheme of the promethazine oxidation by acidified cerium (IV)

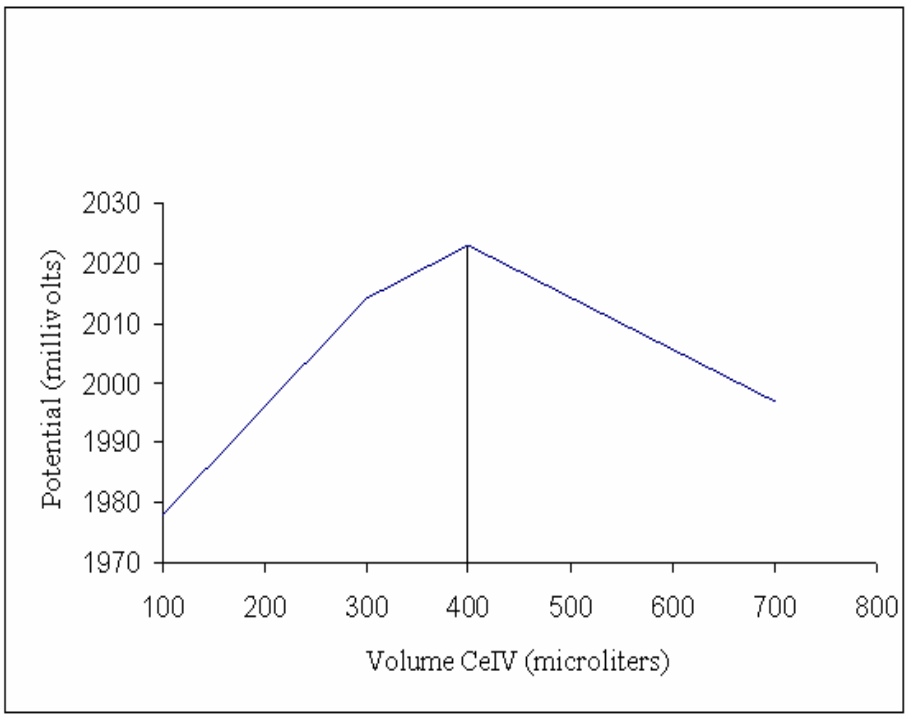


Fig.3.24: DEP titration curve of 1 ml of 3.8×10^{-4}mol l^{-1} Histolac tablets with 1×10^{-3}mol $l^{-1}$of Ce (IV) and 2ml of 1×10^{-3}mol l^{-1} H_2SO_4

3.3.3 Application

The DEP system for the assay of promethazine hydrochloride in pharmaceutical formulations collected from drug stores was successfully applied under the optimum conditions and validated in order to evaluate the precision, recovery, repeatability and accuracy of the method. The optimum conditions of supporting electrolyte concentration ranges from 1×10^{-3}mol l^{-1}- 1×10^{-2}mol l^{-1}, a DC current of 5μA, 20 μl s^{-1} flow rate and cerium IV concentration ranges from 5×10^{-5}mol l^{-1}-1×10^{-3}mol l^{-1} .The mean recovery and the R. S. D. (relative standard deviation) are presented in table 3.11. The results obtained show acceptable precision, accuracy and reproducibility.

Table 3.11 Results obtained by DEP and BP methods for the analysis of promethazine in standard samples and tablets

Promethazine sample	DEP	BP	Official range* %	Recovery %	R. S. D.♠ %
Standard sample	100%	98%	99-101%	102%	± 2%
Histolac 25 mg tablets	95%	95.7%	95-105%	99.2%	± 0.7%

*The official range of the content of a drug specified by the BP.
♠The R. S. D. of n =5

Conclusion

The sequential injection analysis (SIA) technique has been successfully employed for developing new methods for the assay of chlorpheniramine maleate and verapamil hydrochloride in pharmaceutical preparations with.

- The construction of SIA manifolds suitable for the assay of some pharmaceuticals like chlorpheniramine and verapamil.
- Appropriate programming of SIA software to effectively control the newly adopted methods.
- Sequential chemometric optimization of the methods for the assay of chlorpheniramine and verapamil was successfully applied by the employment of the response surface plot and factorial design.
- The newly adopted methods were validated compared to those methods of the BP.
- The proposed SIA methods were successfully applied to pharmaceutical samples as well as for synthetic ones.
- The performance of the newly adopted methods was studied and evaluated with respect to linearity, repeatability, reliability, selectivity, robustness, accuracy and precision.
- The newly adopted methods have some advantages compared to that of the official method in being:
    a) Fully automated.
    b) Time saving.
    c) Economical, with respect to the consumption of reagents.
    d) Safe, with respect to solution handling and to the environment.

For electrochemical method applied for the assay of promethazine hydrochloride in pharmaceutical formulations the following should be noted.

- The system used was easy to construct and simple to operate.
- The program controlling pump was simple with clear and direct commands to run the titration smoothly.

- The method basically relies on the oxidation of promethazine with CeIV in acidic medium, and the detection of the endpoint potentiometirically.
- The detection of the endpoint was based on applying DEP (differential electrolytic potential) theory which was novel.
- The newly adopted method was validated compared to that of the BP.
- The proposed electrochemical method was successfully applied to pharmaceutical samples as well as for synthetic ones.
- The newly adopted method has the advantages of being automated, safe and economical.
- The performance of the method was studied and evaluated with respect to selectivity, sensitivity, accuracy and precision.

Recommendations

In pharmaceutical laboratories where quality control and assurance take place; a huge amount of chemical waste is generated and accumulated day by day. This may eventually lead to harmful consequences on the environment and in turn on humans where personal safety is critically affected. In addition, minimizing of operational costs necessitates the introduction of new methods to be applied with great success along with the conventional analytical procedures, but with many advantages over them. This situation implies intensifying the research in this vital field of sciences. The SIA methodology as a modern analytical technique, can contribute largely to solve many of the problems of traditional analytical laboratories such as waste minimization, cost-effectiveness, speed and automation. Pharmaceutical laboratories can make use of this technology and be applied for routine analysis. The introduction of SIA technique to the analytical chemistry few years ago still being unexplored and a lot have to be done to validate its potentiality in the area of titrimetry.

The adopted SIA titrimetric methods for the assay of chlorpheniramine and verapamil in pharmaceutical formulations have good performance characteristics such as accuracy and precision as well as automation with respect to conventional titrimetric ones. SIA versatility allows almost all titrimetric approaches such as spectrophotometric and potentiometric to be conducted successfully. Hence, it is recommended to adapt SIA technique to conduct the official other than the non-official standard titrimetric methods.

The results of present adopted SIA methods for the assay of chlorpheniramine and verapamil in pharmaceutical formulations showed that experimental design and variable screening can substantially reduce the time needed for method development. So it is recommended to employ chemometrics approach in the development of SIA method.

As far as the electrochemical determination of promethazine hydrochloride is concerned, it is worthy to mention that the application of novel (DEP) detection method was simple, sensitive, economical, and easy to perform. Unlike normal potentiometric it is easy to detect the end point directly from the

DEP titration curves. More work need to be done for more improvement since only a few researchers deal with this vital topic. Hence, it is recommended to adapt the newly introduced DEP method for the determination of pharmaceuticals.

References

1. J. Ruzicka, E. H. Hansen, Flow injection analysis: Part 1. A new concept of fast continuous flow analysis. Anal. Chim. Acta 78(1) (1975) 145-157.
2. K.K. Stewart, G. R. Beecher, P. E. Hare, Anal. Biochem. 70(1976) 167-173.
3. J. Ruzicka, Flow-injection Methods: A new tool for instrumental analysis. Philosophical Transations R. Soc. Lond. Series A, Math. Phys. Sci. 305(1491) (1982) 645-656
4. J. Ruzicka, E. H. Hansen, Flow injection analysis, 2nd edition. Willey, New York (1988)
5. M. Valcárcel, M. D. Luque de Castro, Flow-injection analysis: Principles and Applications, Ellis Holwood, Chichester, 1987.
6. B. Karlberg, G. E. Pacey, Flow-injection analysis: A Practical Guide, Techniques and Instrumentation in Analytical Chemistry, Elsevier Amsterdam, 1989.
7. J. H. Aldstadt, D. C. Olson, D. K. Wolcott, G. D. Marshal, S. W. Steig, Flow and sequential injection analysis techniques in process analysis. In R. A. Meyers, R. A. Meyers Encyclopedia of Analytical Chemistry Volume 15: Applications, Theory and Instrumentation, John Wiley & Sons Inc (2000).
8. J. Ruzicka, G. D. Marshal, Sequential injection: A new concept for chemical sensors, process analysis and laboratory assays. Anal. Chim. Acta. 237(2) (1990) 329-343
9. J. Ruzicka, E. H. Hansen, Flow injection analysis-Where are we heading? Trends Anal. Chem. 17(2) (1998) 69-73.
10. M. W. Barnett, C. E. Lenehan, and S. W.Lewis, Sequential injection analysis: an alternative approach to process analytical chemistry. Trends Anal. Chem. 18(5) (1999) 346-352.
11. J. Ruzicka, Lab-on-valve: Universal microflow analyzer based on sequential and bead injection. Analyst 125(6) (2000) 1053-1060.

12. Fletcher P., Andrew K. N., Calokerinos A. C., Forbes S., Worsfold P. J., Analytical applications of flow injection with chemiluminiesnce detection-a review. Luminesence 16(1) (2001) 1-23.
13. A. Wolf, R. Weber, R. Huttl, J. Lerchner, G. Wolf, Sequential flow injection analysis of complex systems using calorimetric detection. Thermochimica Acta 382 (1-2) (2002) 89-98.
14. N. Piza, M. Armas, B. E. Garciela, J M. Estela, V. Cerda, Implementation of chemiluminescence detection in the multisyringe flow injection technique. Anal. Chim. Acta. 467(1-2) (2002) 155-166.
15. E. H. Hansen, The impact of flow injection on modern chemical analysis: Has it fulfilled our expectations? And where are we going? Talanta 64 (5) (2004) 1076-1083.
16. Abu baker M. Idris, Salah M. Sultan, Kamal E. Ibrahim, Fahad N. Assubaie, Sequential injection spectrophotometric kinetic method for the quantitative determination of Paracetamol in dosage forms.
17. J. F. van Staden, R. J. Stefan, sequential injection analysis Anal. Bioanal. Chim. 374(2002) 3-12.
18. J. F. van Staden, R. E. Taljaard, On-line dilution with sequential injection analysis a system for monitoring sulphate in industrial effluents, Fresenius J. Anal. Chem. 357(1997) 577-581.
19. A. Baron, M. Guzmain, J. Ruzicka, G. D. Christain, Single standard calibration and dilution by sequential injection analysis technique, Analyst. 117(1992) 1839-1844.
20. K. L. Peterson, B. K. Logan, G. D. Christian, J. Ruzicka, sequential injection for sample preparations, Anal. Chim. Acta. 337 (1997) 99-103.
21. F. V. Silva, A. R. A. Nogueria, G. B. Souza, G. M. Cruz, Sequential injection analysis a useful tool for on-line sample handling and pretreatment, Anal. Sci. 16 (2000) 361-364.
22. J. Wang, E. H. Hansen, Sequential injection lab-on-valve: The third generation of flow injection analysis. Trends Anal. Chem. 22(4) (2003) 225-231.

23. J. Wang, E. H. Hansen, The three generations of flow injection analysis. Anal. Lett. 37(3) (2004) 345-359.
24. Petr Chocholousˇ, Petr Solich, Dalibor Sˇ atı´nsky, An overview of sequential injection chromatography, analytica chimica acta 600 (2007) 129–135.
25. S. K. Hartwell, G. D. Christin, K. Grudpan, Bead injection with a simple flow injection system: An economical alternative for trace analysis. Trends Anal. Chem. 23(9) (2004) 619-623.
26. C. E. Lenhen, N. W. Barnett, S. W. Lewis, Sequential injection analysis. Analyst (Cambridge, UK) 127(8) (2002) 997-1220.
27. T. Gubeli, G. D. Cristin, J. Ruzicka, Fundamentals of sinusoidal flow sequential injection spectrophotometry. Anal. Chem. 63(21) (1991) 2407-2413
28. G. D. Marshal, J. F. van Staden, Operational parameters affecting zone penetration in sequential injection analysis. Process Cont. Qual. 3(1-4) (1996) 251-262
29. A. Cladera, E. Gomez, J. M. Estela, V. Cerda, Effect of variables influencing $S_{1/2}$ in sequential injection analysis. Extraprobability of $S_{1/2}$ based results between SIA designs. Talanta 43(10) (1996) 1667-1674.
30. J. M. Calatayud, Flow injection analysis of pharmaceuticals. Taylor Francis, London, UK (1996).
31. P. Solich, H. Sklenarova, M. Polasek, R. Larlicek, Application of flow injection technique in pharmaceutical analysis. Part I: Spectrophotometric and chemiluminescence detection. J. Flow injection Anal. 18(2) (2001)1-13.
32. P. Solich, H. Sklenarova, M. Polasek, R. Larlicek, Application of flow injection technique in pharmaceutical analysis. Part II: Other spectroscopic methods and electroanalytical detection. J. Flow injection Anal. 18(2) (2001) 118-132.

33. P. Solich, M. Polasek, J. Kilmundova, J. Ruzicka, Sequential injection technique applied to pharmaceutical analysis. Trends Anal. Chem. 23(2) (2004) 116-126.
34. A. M. Pimenta, M. C. B. S. M. Montengro, A. N. Araújo, J. Martinez Calatayud, Application of sequential injection analysis to pharmaceutical analysis. J. Pharma. Biomed. Anal. 40 (2006) 16-34.
35. P. D. Tzanavaras, C. K. Zacharis, G. A. Theodoidis, E. A. Kalaitzantonakis, A. N. Voulgaropoulos, Normal spectrophotometric and stopped-flow spectroflourimetric sequential injection methods for the determination of Alendronic acid in pharmaceuticals, Talanta 547 (2005) 98-103.
36. D. Satinsky, L. M. L. Santos, H. Sklenarova, P. Solich, M. C. B. S. M. Montengro, A. N. Araújo, Sequential injection chromatographic determination of Ambroxol and doxycycline in pharmaceutical preparations, Anal. Chim. Acta. 68 (2005) 214-218.
37. A. Chisvert, J. V. Izquierdo, A. Salvador, A sequential injection system for spectrophotometric determination of p-aminobenzoic acid in sunscreens, Anal. Bioanal. Chem. 374 (2002) 963-967.
38. A. Salvador, A. Chisvert, A. Rodriquez, J. G. March, Indirect sequential injection system for spectrophotometric determination of p-aminobenzoic acid in sunscreens, Anal. Chim. Acta. 493 (2003) 233-239.
39. P. C. A. G. Pinto, M. L. M. F. S.Saraiva, J. L. M. Santos, J. L. F. C. Lima, Flourimetric determination of amincaproic acid in pharmaceutical formulations using sequential injection analysis system, Talanta.39(68)(2006) 857-862.
40. A. Pasamontes, M. P. Callao, Determination of amoxycillin in pharmaceuticals using sequential injection analysis: evaluation of the presence of interferents using multivariate curve resolution, Anal. Chim. Acta. 485 (2003) 195-204.

41. A. Pasamontes, M. P. Callao, Determination of amoxycillin in pharmaceuticals using sequential injection analysis and multivariate curve resolution, Anal. Chim. Acta. 485 (2004) 159-165.
42. H. Paseková, M. Polásek, Determination of procaine, benzocaine and tetracaine by sequential injection analysis with permanganate-induced chemiluminescence detection. Talanta 52(1) (2000) 67-75.
43. A. Chisvert, M. T. Vidal,A. Salvador, Sequential injection analysis for benzophenone-4 and phenyl-benzimidazole sulphonic acid in sunscreen sprays by solid phase extraction coupled with ultraviolet spectrometry, Anal. Chim. Acta. 464 (2002) 295-301.
44. P. D. Tzanavaras, D. G. Themelis, A. Economou, Sequential injection method for the direct spectrophotometric determination of bismuth in pharmaceutical products, Anal. Chim. Acta. 505 (2004) 167-171.
45. D. Satinsky, H. Sklenarova, J. Huclova, R. Karlicek, Determination of bopindolol by sequential injection technique with spectrophotometric detection, ll Fármaco 58 (2003) 1057-1062
46. J. F. van Staden, M. Tsanwani, Determination of Boron as boric acid in eye lotions using a sequential injection system. Talanta 58(6) (2002) 1103-1108
47. A. Economou, D. G. Themelis, H. Bikou,P. D. Tzanavaras, P. G. Rigas, Determination of boron in water and pharmaceuticals by sequential injection analysis and fluorimetric detection, Anal. Chim. Acta. 510 (2004) 219-224.
48. S. M. Sultan, F. E. O. Suliman, Use of sequential injection technique for mechanistic studies and kinetic determination of bromazepam complexed with iron (II) in hydrochloric acid. Analyst (Cambridge, UK) 121(5) (1996) 617-621.
49. S. M. Sultan, Y. A. M. Hassan, K. E. E. Ibrahim, Sequential injection Spectrophotometric assay of bromazepam complexed with iron (II) in hydrochloric acid with chemometric optimization. Talanta 50(4) (1999) 841-849.

50. J. F. van Staden, R. E. Taljaard, Determination of calcium in water, urine and pharmaceutical samples by sequential injection analysis. Anal. Chim. Acta. 323(1-3) (1996) 75-85.
51. A. M. Pimenta, A. N. Araújo, M. C. B. S. M. Montengro, Sequential injection analysis of captopril based on colorimetric and potentiometric detection, Anal. Chim. Acta. 438 (2001) 1-38.
52. P. D. Tzanavaras, D. G. Themelis, A. Economou, G. Theodoridos, Flow and sequential injection manifolds for the spectrophotometric determination of captopril based on its oxidation by Fe (III), Microchim Acta 142 (2003) 55-62.
53. F. E. O. Suliman, S. M. Sultan, Sequential injection technique employed for stoichiometric studies, optimization and quantitative determination of some fluoroquinolone antibiotics complexed with iron (III) in sulfuric acid media. Talanta 43(4) (1996) 559.
54. A. M. Pimenta, A. N. Araújo, M. C. B. S. M. Montengro, Simultaneous potentiometric and fluorimetric determination of Diclofenac in sequential injection analysis system, Anal. Chim. Acta. 470 (2002) 185-194.
55. N. W. Beyene, J. F. van Staden, R. J. Stefan, Sequential injection spectrophotometric determination of etilefrine hydrochloride, ll Fármaco 59 (2004) 1005-1010.
56. N. W. Beyene, J. F. van Staden, R. J. Stefan, Determination of fenoterol hydrobromide by sequential injection analysis with spectrophotometric detection, Anal. Chim. Acta. 521 (2004) 223-229.
57. P. C. A. G. Pinto, M. L. M. F. S.Saraiva, J. L. M. Santos, J. L. F. C. Lima, A pulsed sequential injection analysis flow system for the fluorimetric determination of indomethacin in pharmaceutical preparations, Anal. Chim. Acta. 539 (2005) 173-179.
58. J. A. Erustes, R. Forteza, V Cerda, Sequential injection procedure for the determination of iodide in pharmaceutical and drinking water samples by catalytic reaction with Spectrophotometric detection. J. A. O. A. C. Int. 84(2) (2001) 337-341.

59. Z. O. Telfadest, J. F. van Staden, R. J. Stefan, Sequential injection determination of trace amounts of iodide by its catalytic effect on the 4,4-methylenebis (N,N-dimethylanaline)-chromine-T reaction, Talanta 64 (2004) 1213-1219.
60. J. F. van Staden, H. du Plessis, R. E. Taljaard, Determination of iron (III) in pharmaceutical samples using dialysis in sequential injection analysis system. Anal. Chim. Acta. 357(1-2) (1997) 141-149.
61. P. C. C. Oliveira, J. C. Masini, Sequential injection determination of iron(III) in anti-anemic pharmaceutical formulations with Spectrophotometric detection Anal. Lett. 34(3) (2001) 389-397.
62. J. F. van Staden, E. B. Naidoo, Determination of iron as Fe (II) in multivitamins, haemataenics and effluent streams using solid-phase (cadmium reactor) incorporated in sequential injection system, S. Afr. J. Chem. 53 (2000) 3-17.
63. Z. O. Telfadest, J. F. van Staden, R. J. Stefan, Sequential injection spectrophotometric determination of iron as Fe (II) in multivitamin preparations using 1,10-phenanthroline as complexing agent, Talanta 64 (2004) 1189-1195.
64. L. V. Mulauzadi, J. F. van Staden, R. J. Stefan, On-line determination of iron (II) and iron (III) using spectrophotometric sequential injection system, Anal. Chim. Acta. 467 (2002) 35-49.
65. N. W. Beyene, J. F. van Staden, R. J. Stefan, H. Y. Aboul-Enein, Determination of isoxsuprine hydrochloride by sequential injection visible spectrophotometry, ll Fármaco 60 (2005) 613-619.
66. C. K. Zacharis, P. D. Tzanavaras, D. G. Themelis, G. Theodoridos, A. Economou, P. G. Rigas, Rapid spectroflourimetric determination of lisinopril in pharmaceutical tablets using sequential injection analysis, Anal. Bioanal. Chem. 379 (2004) 759-763.
67. Z. O. Telfadest, J. F. van Staden, R. J. Stefan, Spectrophotometric determination of magnesium in pharmaceutical preparations by cost-effective sequential injection analysis, Talanta 64 (2004) 981-988.

68. J. Fang, A. Wang, S. Feng, J. wang, Non-equilibrium determination of metoclopramide and tetracaine hydrochloride by sequential injection spectrophotometry, Talanta 66 (2005) 236-243.
69. E. P. Zisiou, P. C. A. G. Pinto, M. L. M. F. S.Saraiva, C. Siquet, J. L. F. C. Lima, Sensitive sequential injection determination of naproxen based on interaction with β-cyclodextrin, Talanta 68 (2005) 226-230.
70. A. Chisvert, A. Salvador, M. C. Pascual-Marti, J. G. March, Efficient flow injection and sequential injection methods for spectrophotometric determination of Oxybenzone in sunscreens based on reaction with Ni (II), Fresenius J. Anal. Chem. 369 (2001) 684-689.
71. A. Salvador, A. Chisvert, A. Camarasa, M. C. Pascual-Marti, J. G. March, Sequential injection spectrophotometric determination of Oxybenzone in lipsticks, Analyst 126 (2001) 1462-1465.
72. F. E. O. Suliman, S. M. Sultan, Sequential injection method for the determination of oxprenolol in pharmaceutical products using chemometric methods of optimization. Microchem. J. 57(3) (1997) 320-327.
73. J. F. van Staden, M. Tsanwani, Determination of paracetamol in pharmaceutical formulations using sequential injection system.Talanta.58 (6) (2002) 1095-1101.
74. R. Burakham, S. Duangthong, L. Patimapornlert, N. Lenghor, S. Kasiwad, L. Srivichai, S. Lapanantnoppkhum, J. Jakmunee, K. Grudpan, Flow injection and sequential injection determination of Paracetamol in pharmaceutical preparations using nitrosation reaction, Anal. Sci. 20 (2004) 837-840.
75. S. M. Sultan, F. E. O. Suliman, B. B. Saad, Use of the sequential injection technique to determine the concentration and stoichiometries of trimperazine and perphenazine complexed with palladium (II) in hydrochloric acid. Analyst (Cambridge, UK) 120(2) (1995) 561-564.
76. S. M. Z. Al-kindy, A. Al-Wishahi, F. E. O. Suliman, A sequential injection method for the determination of piroxicam in pharmaceutical formulations using europium sensitized fluorescence, Talanta 64 (2004) 1343-1350.

77. S. M. Sultan, N. I. Desai, Sequential injection analysis technique for the concentration, stoichiometry and formation constant studies of promethazine hydrochloride complexed with palladium (II) in hydrochloric acid. Analyst (Cambridge, UK) 122(9) (1997) 911-914.
78. S. M. Sultan, Y. A. M. Hassan, A. M. Abulkibash, Chemiluminescence assay of promethazine hydrochloride using acidic permanganate employing flow injection mode operated with syringe and peristaltic pumps. Talanta 59(6) (2003) 1073-1080.
79. H. Paseková, M. Polásek, J. F. Cigaro, J. Dolejsová, Determination of some sulphonamides by sequential injection analysis with chemiluminescence detection. Anal. Chim. Acta. 438(1-2) (2001) 165-173.
80. M. Polásek, M. Jamber, Chemiluminescence determination of antibacterial drug trimethoprim by automated sequential injection analysis technique with permanganate and hexametaphosphate as reagents. Talanta 58(6) (2002) 1253-1261.
81. L. X. Tang, F. J. Roeel, Rapid determination of Warfarin by sequential injection analysis with cyclodextrin enhanced, Anal. Lett. 31 (1998) 891-901.
82. H. Zhu, H. Chen, Y. Zhu, Determination of thiamine in pharmaceutical preparations by sequential injection renewable surface solid-phase spectrophotometry, Anal. Sci. 19 (2003) 289-294.
83. S. M. Sultan, N. I. Desai, mechanistic study and kinetic determination of vitamin C employing sequential injection technique, Talanta 45 (1998) 1061-1071.
84. S. M. Sultan, Y. A. M. Hassan, K. E. E. Ibrahim, Sequential injection technique for automated titration: Spectrophotometric assay of vitamin C in pharmaceutical products using cerium (IV) in sulfuric acid. Analyst 124(6) (1999) 917-921.
85. N. Lenghor, J. Jakmunee, M. Vilen, R. Sara, G. D. Christian, K. Grudpan, Sequential injection redox or acid-base titration for determination of ascorbic acid or acetic acid. Talanta 58(6) (2002) 1139-1144.

86. D. T. Newcombe, T. J. Cardwell, R. W. Cattrall, S. D. Kolev, An optical membrane redox chemical sensor for the determination of Ascorbic acid. Lab. Rob. Autom. 12 (2000) 200-204.
87. D. T. Newcombe, T. J. Cardwell, R. W. Cattrall, S. D. Kolev, An optical redox chemical sensor based on ferroin immobilized in Nafion® membrane. Anal. Chim. Acta. 401(1-2) (1999) 137-144.
88. J. F. van Staden, M. Tsanwani, Determination of zinc in pharmaceutical products by use of sequential injection analysis system. J. Anal.Chem.371 (3) (2001) 376-379.
89. R. J. Stefan, R. G. Bokrestsion, J. F. van Staden, H. Y. Aboul-Enein, Immunosensor for the determination of azidothymidine: Its utilization as a detector in sequential injection analysis. Talanta 59(5) (2003) 883-887.
90. R. J. Stefan, J. F. van Staden, H. Y. Aboul-Enein, Amperometric biosensors/sequential injection analysis system for simultaneous determination of S- and R- captopril, Biosens. Bioelectron. 15 (2000) 1-5.
91. R. J. Stefan, J. F. van Staden, H. Y. Aboul-Enein, Simultaneous detection of S and R captopril using sequential injection analysis. Talanta 51(5) (2000) 969-975.
92. R. J. Stefan, J. F. van Staden, H. Y. Aboul-Enein, On-line assay of S-captopril using an amperometric biosensor / sequential injection analysis Anal. Chim. Acta. 411(1-2) (2000) 51-65.
93. A. M. Pimenta, A. N. Araūjo, M. Conceicao, B. S. M. Montenegro, Sequential injection analysis of captopril based on colorimetric and potentiometric detection. Anal. Chim. Acta. 438(1-2) (2001) 31-38.
94. A. M. Pimenta, A. N. Araūjo, M. Conceicao, B. S. M. Montenegro, C. Pasquinini, J. J. R. Rohwedder, I. M. Raimundo Jr., J. Pharm. Biomed. Anal. 36 (2004) 49-55.
95. A. M. Pimenta, A. N. Araūjo, M. Conceicao, B. S. M. Montenegro, Sequential injection analysis system for potassium clavulanate determination using two potentiometric detectors. J. Pharam. Biomed. Anal. 30(4) (2002) 931-937.

96. A. M. Pimenta, A. N. Araūjo, M. Conceicao, B. S. M. Montenegro, Increasing the scope and power of flow-injection analysis through chemometric approaches. Anal. Chim. Acta. 470(2) (2002) 185-194.
97. R. J. Stefan, J. F. van Staden, C. Bala, H. Y. Aboul-Enein, On-line assay of the S-enantiomers of enalapril, ramipril and pentopril using sequential injection analysis/amperometric biosensor system, J. Pharm. Biomed. Anal. 36 (2004) 889-892.
98. E. M. G. Santos, A. N. Araūjo, C.M.C. M Couto, B. S. M. Montenegro, A. Kejzlarova, P. Solich, Ion-selective electrode for penicillin-G based on Mn (III) TPP-CI and their application in pharmaceutical formulations controlled by sequential injection analysis, J. Pharm. Biomed. Anal. 36 (2004) 701-709.
99. R. J. Stefan, J. F. van Staden, H. Y. Aboul-Enein, Simultaneous determination of l-throxine (l-T_4), d-throxine (d-T_4) and l-triiodothyronine (l-T_3) using sensors/ sequential injection analysis system, Talanta 64 (2004) 151-155.
100. W. W. Kubiak, R. M. Latonen, A. Ivaska, Sequential injection analysis system with adsorptive stripping voltammetric detection. Talanta 53(6) (2001) 1211-1219.
101. R. N. Fernandes, M. G. F. Sales, B. F. Reis, E. A. G. Zagatto, A. N. Araūjo, M. Conceicao, B. S. M. Montenegro, LC determination of the enantiometric purity of levamisole using stationary phase with bonded naphthylcarbamoyated-β-cyclodextrin. J. Pharm. Biomed. Anal. 25(3-4) (2001) 407-415.
102. R. W. Min, J. Nielson, J. Villadsen, Simultaneous monitoring of glucose, lactic acid and penicillin by sequential injection analysis, Anal. Chim. Acta 312 (1995) 149-156.
103. X. Z. Lui, Z. L. Fang, Sequential injection system for drug-dissolution studies of ibuprofen tablets and sustained released formulations, Anal. Chim. Acta 358 (1998) 103-110.

104. C. M. Schulz, J. Ruzicka, Real-time determination of glucose consumption by live cells using lab-on-valve sequential injection analysis with integrated microbioreactors, Analyst 127 (2002) 1293-1298.
105. D. L. Massart, B. G. M. Vandeginste, L. M. C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Data Handling in science and technology 20A: Handbook of chemometrics and qualimetrics, Part A, Elsevier, Oxford, p.1 (1997).
106. B. Karlberg, R. Torgrip, Increasing the scope and power of flow injection analysis through chemometric approaches. Anal. Chim. Acta. 500(1-2) (2003) 299-306.
107. D. C. Montgomry, Design and analysis of experiments. 2nd edition, Wiley, New York (1996).
108. Myers R. H., Response surface methodology. Allyn and bacon, Boston (1971).
109. E. D. Morgan, chemometrics: Experimental design. ACOL, London and John Wiley and sons, England (1991).
110. R. G. Brereton, chemometrics: Data Analysis for the Laboratory and chemical plant, Wiley (2003).
111. H. Marten, T. Naes, Multivariate calibration, Wiley (1989).
112. Multisimplex, User's guide to Multisimplex® 2.1, Grabitech solutions AB Sendsvell, Sweden (2000).
113. S. M. Sultan, A. M. S. Abdenabi, A. m. Almuaibed, Chemiluminescence method for the assay of perphenazine in drug formulation using permanganate in sulfuric acid with flow injection technique and a chemometrical approach. Talanta 49 (4) (1996) 1051-1057.
114. F. O. suliman, S. M. Sultan, sequential optimization of flow injection spectrophotometric method for the assay of chlorpromazine in pharmaceutical preparations. Talanta 41 (11) (1994) 559-568.
115. S. M. Sultan, A. m. Almuaibed, A. Townshend, Flow injection chemiluminescence determination of medazepam. Fresenius J. Anal. Chem. 362 (1998) 167-169.

116. S. M. Sultan, A. D. Walmsley, Chemometrical optimization, FIA of perphenazine assay. Talanta 46(5) (1998) 897-906.
117. S. M. Sultan, F. O. suliman, Chemometric optimization and flow injection method for the determination of norfloxacin in drug formulations. Analyst 118(5) (1993) 573-576.
118. S. M. Sultan, F. O. suliman, Application of super modified simplex optimization to flow injection spectrophotometric determination of Promethazine hydrochloride in drug formulations. Anal. Sc. 8(6) (1992) 841-843.
119. S. M. Sultan, F. O. suliman, Flow injection method for the assay of antiarrythmic drug procainamide hydrochloride in drug formulations utilizing statistical optimization techniques. Talanta 40(5) 1993) 623-627.
120. S. M. Sultan, F. O. suliman, Flow injection spectrophotometric determination of the antibiotic ciprofloxacin in drug formulations. Analyst 117(9) (1992) 1523-1526.
121. S. M. Sultan, F. O. suliman, O.Duffuaa, I. I. Abu-Abdoun, Simplex-optimized and flow injection spectrophotometric assay of tetracycline antibiotics in drug formulations. Analyst 117(9) (1992) 1179-1183.
122. Ocean optics, Inc. E-copy on CD version 060203 (2003).
123. BP, E-copy on CD version 6 (2002).
124. USP, E-copy on CD, Version 26-NF 21(2003).
125. Lingane, James, J. Electrochemical Chemistry second edition Interscience Publisher, INC. NEW YORK.
126. Dutoit, P. and Weise, G. J. Chem. Phys. 578, 1911, 608.
127. Willard, H. H. and Fenwich, F., J. Am. Chem. Soc. 44, 1922, 2504.
128. Bishop, E., Differential electrolytic potentiometry part II: the precision and accuracy of applications to redox titrimetry, Analyst (London), 83, 1958, 212.
129. Bishop, E., short, G.D, Differential electrolytic potentiometry part VI: the precision and accuracy of applications to acid-base titration with antimony electrodes, Analyst, 87, 1962, 467.

130. Bishop, E. R., Dhaneshwer, B. Differential electrolytic potentiometry part VI: The behavior and energetics of current-carrying silver and silver halide electrodes in the semi-micro-scale titration, Analyst 87, 1962, 845.
131. Bishop, E., Dhaneshwer, R. B., Anal.Chem. 36, 1964, 726.
132. Bishop, E., Mikrochim. Acta, 40, 1956, 619.
133. Bishop, E., Analyst, 83, 1958, 212.
134. Bishop, E., Analyst, 83, 1960, 422.
135. Malmstadt, H., V. E. R., Fett, Anal. Chem., 27, 1955, 1757.
136. Monk, R., Steed, G. K. C. Anal. Chim. Acta 26, 1962, 305.
137. Abulkibash, A. M., Sultan, S. M., Al-Olyan, A.M., Alghannam Sh.M., Talanta 61, 2003, 239.
138. Abdennabi, A.M.S., Bishop, E., Analyst 107, 1982, 1032.
139. Bishop, E., Abdennabi, A.M.S., Analyst 108, 1983, 1349.
140. Abdennabi, A.M.S. Bishop, E., Analyst 108, 1983, 71.
141. Abdennabi, A.M.S., Rashed, M., AJSE 12, 1986, 82.
142. Abdennabi, A.M.S., Bishop, E., Analyst 108, 1983, 1227.
143. K. Basavaiah, J. M. Manjunatha, G. Krishnamurthy, Quantitation of pharmaceutically important phenothiazines by oxidmetry. Il farmaco 55(2000) 87-92.
144. H. S. Gowda, S. A. Ahmed, N-subistituted phenothiazines as indicators in titrations with chloramines-T. Anal. Chim. Acta. 99(2) (1978) 343-349.
145. K. Basavaiah, J. M. Swamy, Titrimetric micro determination of some phenothiazine neuroleptics with potassium hexacynoferrate. Talnta 47(1) (1998) 59-66.
146. Bhongade SL, Kasture AV, Spectrophotometric determination of phenothiazine derivatives. Indian J. Pharm. Sci. 55(4) (1993) 151-154.
147. K. Basavaiah, J. M. Swamy, Application of potassium dichromate and iron thiocyanate in the spectrophotometric investigations of Phenothiazines Il Farmaco 56(8) (2001) 579-585.

148. K. Basavaiah, J. M. Swamy, A highly sensitive spectrophotometric method for the determination of some phenothiazine antipsychotic drugs using Chloramines-T and indigocarmine. Anal. Sci. 17(8) (2001) 963-967.
149. K. Basavaiah, J. M. Swamy, Titrimetric and spectrophotometric determination of some phenothiazine psycotopics in pure form and in pharmaceutical formulations with metavandate. Microchim. Acta. 137(1-2) (2001) 75-80
150. E. regulska, M. Tarasiewicz, H. Puzanowska-Tarasiewcz, Extractive-spectrophotometric determination of some phenothiazines with dipicrylamino acid. J. Pharm. Biomed. Anal. 27(1) (2002) 335-340.
151. K. Basavaiah, Determination of some psycotopic phenothiazine drugs by charge transfer complexation reaction with chloranilic acid. Il Farmaco 59(4) (2004) 315-321.
152. M. J. Saif, J. Anwar, A new spectrophotometric method for the determination of some promethazine-HCl from pure and in pharmaceutical preparations Talanta. 67(5) (2005) 869-872.
153. T. Aman, J. Anwar, A. Ahmed, L. Latif, Determination of five phenothiazines in pure pharmaceutical preparations using vanadium pentoxide as chromogenic reagent. Anal. Lett. 36(14) (2003) 2961-2974.
154. E. O. Fadiran, A. G. Davidson, Determination of low levels of phenothiazine sulphoxides in phenothiazine drug substances and formulations by thin-layer chromatography-second derivative spectrofluorimetry. J. Chromatograph. A 442 (1988) 363-370.
155. A. N. Díaz, Fiber-optic remote sensor for in situ fluorimetric quantification in thin-layer chromatography. Anal. Chim. Acta. 225(2) (1991) 297-303.
156. L. Pena, A. Gómez-Hens, D. Pérez-Bendito, Kinetic fluorimetric determination of promethazine by stopped-flow mixing technique, J. Pharm. Biomed. Anal. 11(10) (1993) 893-896.
157. I. Hornyak, L. Kozma, A. Lapat, I. Tovari, Spectrofluorimetric determination of diethazine and promethazine in pharmaceutical preparations. Biomed. Chromatogra. 11(2) (1997) 99-101.

158. A. A. Alwarthan, S.A. Al-Tamarah, A. A. Akel, Determination of promethazine by its inhibition of the chemiluminescence of luminal-hydrogen peroxide-chromium(III) system. Anal. Chim. Acta. 282(1) (1993) 169-174.
159. A. R. Fox, D. A. McLoughlin, Rapid and sensitive high-performance liquid chromatographic method for the quantification of promethazine in human serum with electrochemical detection. J. Chromatogr. A. 631(1-2) (1993) 225-259.
160. G. Taylor, J. B. Houston, Simultaneous determination of promethazine and two of its circulating metabolites by high-performance liquid chromatography. J. Chromatogr. B: Bimed Sci Appl. 230(1) 194-198.
161. R. I. Baxter, G. S. B. Kerr, A. D. Woolfson, Determination of promethazine by anodic differential-pulse voltammetry. Anal. Chim. Acta. 164 (1984) 171-180.
162. Y. Ni, L. Wang, S Kokot, Voltammetric determination of chloropromazine hydrochloride and promethazine hydrochloride with the use of multivariate calibration. Anal. Chim. Acta. 439 (1) (2001) 159-168.
163. S. M. Golbali, M. Showkati-Shishevan, Potentiometric titration of phenothiazine compounds in chloroform and its use in pharmaceutical analysis. Talanta 38(11) (1991) 1253-1256.
164. J. L. F. C. Lima, M. C. B. S. M. Montengro, M. G. F. Sales, ion-selective electrodes for the determination of promethazine in pharmaceutical preparations and application to flow injection analysis. J. Pharm. Sci 86(11) (1997) 1234-1238.
165. P. G. H. M. Muijselaar, H. A. Claessens, C. A. Cramers, Determination of structurally related phenothiazines by capillary zone electrophoresis and micellar elecrokinetic chromatography. J. Chromatogr. A 735(1-2) (1996) 395-402
166. R. Wang, X. Lu, M. Wu, E. Wang, Separation of promethazine and thioridazine using capillary electrophoresis with end-column amperometric detection. J. Chromatogr. B: Bimed Sci Appl. 721(2) (1999) 327-332.

167. W. Jin, Q. Xu, W. Li, A new capillary elecrophoresis end-column amperometric detection system without the need for capillary/electrode alignment. Elecrophoresis 21(8) (2000) 1527-1534.
168. R. Wang, X. Lu, M. Wu, Chiral separation of promethazine using capillary electrophoresis with end-column amperometric detection. J. Separation Sci. 24(8) (2001) 658-662.
169. Y. M. Issa, W. F. El-Hawary, A. F. Ahmed, Ion pair formation in pharmaceutical analysis. Conductimetric determination of promazine, chloropromazine, promethazine, imipramine and ciprofloxacin hydrochlorides in pure form, drug formulations and urine. Michrochim. Acta. 134(2000) 9-14.
170. J. M. Calatayud, V. G. Mateo, Cerium (IV) arsenite as a solid-phase reactor for use in flow injection analysis. Spectrophotometric determination of promethazine. Anal. Chim. Acta. 264(2) (1992) 283-289.
171. S. M. Sultan, Computer assisted optimization of a flow-injection method for the assay of chloropromazine, promethazine and trimeprazine in drug formulations. Talanta 40(5) (1993) 37-42.
172. Abubaker M. Idris, Fahad N. Assubaie, Salah M. Sultan, Chemometric optimization of a SIA promethazine hydrochloride assay method. Microchemical journal 83(2006) 7-13.
173. J. M. Calatayud, T. G. Sancho, Spectrophotometric determination of promethazine by flow injection analysis and oxidation by cerium (IV). J. Pharm. Biomed. Anal. 10(1) (1992) 37-42.
174. M. J. R. Rama, A. R. Mrdina, A. M. Díaz, Bead injection spectroscopy-flow injection analysis. (BIS-FIA): An interesting tool applicable to pharmaceutical analysis: Determination of promethazine and trifluperazine J. Pharm. Biomed. Anal. 35(5) (2004) 1027-1034.
175. D. Chen, A. Ríos, M. D. L. Castro, M. Valcárcel, Simultaneous flow-injection determination of chloropromazine and promethazine by photochemical reaction. Talanta 38(11) (1991) 1227-1233.

176. D. Daniel, I. G. R. Gutz, Flow injection spectroelectroanalytical method for determination of promethazine hydrochloride in pharmaceutical preparations. Anal. Chim. Acta. 494(1-2) (2003) 215-224.
177. J. M. Calatayud, S. N. Sarrion, A. S. Sampedro, C. G. Benito, Determination of promethazine hydrochloride with bromophenol blue by a turbidimetric method and flow injection analysis. Microchem. J. 45(2) (1992) 129-136.
178. Martindale: The complete drug reference, thirty fourth edition, The Pharmaceutical press (2005).
179. Einar Jacobsen, Knut Hogberg, Polargraphic determination of chlorpheniramine, J. Anal. Chim. Acta. 71(1) (1974) 157-163.
180. N. B. Pappano, Y. C. D. Micalizzi, N. B. Debattista, F. H. Ferretti, Talanta 44(1997) 633.
181. Nevin Erk, Quantitative analysis of chlorpheniramine and phenylephrine in nasal drops by differential-derivative spectrophotometric, Zero-crossing first derivative UV spectrophotometry and absorbance ratio methods, J. Pharm. Biomed. 23 (6) (2000) 1023-1031.
182. M. Blanco, M. Alcalá, Quantification of five active principles in pharmaceutical preparations by near infra red spectroscopy (development and validation), European J. Pharma. Sci. 27 (2-3) (2002) 280-286.
183. C. Martinz-Algaba, J. M. Bermúdez-Saldaňa, R. M. Villanueva- camaňas,, S.Sagrato, M. J. Medina-Hernández, Analysis of pharmaceutical formulations containing antihistamine drugs by micellar liquid chromatography, J. Pharm. Biomed. 40 (2) (2006) 312-321.
184. Mayte Gil-Agusti, Llorenç Monferrer-Pons, Maria Celia García, Aluarez-Coque, Josep steve-Romero, Determination of active ingredients in cough syrup by micellar Liquid chromatography, Talanta, 54(4) (2001) 621-630.
185. Yingju liu, Wuyi Zhou, Determination of chlorpheniramine and its binding with human serum albumin by capillary electrophoresis. J. Anal. Sci. 22 (2006) 999-1003.

186. M. Elisa Capella-Peiro, Alessandra Bossi, Josep steve-Romero, Optimization by factorial design of capillary zone electrophoresis method for simultaneous separation of antihistamines. J. Anal. Biochem. 352(1) (2006) 41-49.
187. A. Marín, C. Barbas, Dissolution test and determination of pharmaceutical formulations containing chlorpheniramine by capillary electrophoresis and high performance liquid chromatography, J. Pharma. Biomed. 35(4) (2004) 769-777.
188. Yumei Long, Weifeng Li, Lihua Nie, Shouzhuo Yao, Preparation and application of chlorpheniramine ion-selective piezoelectric sensor, J. Anal. Chim. Acta. 395 (1999) 33-40.
189. A. García, F. J. Rubérez, A. Marín, A. de la Maga, C. Barbas, Determination of acetaminophen, phenylephrine and chlorpheniramine in pharmaceutical formulations by HPLC, J. Chromatography B 785(2) (2003) 237-243.
190. A. Marín, E.García, A. García, C. Barbas, Quantification of acetaminophen, phenylephrine and chlorpheniramine pharmaceutical formulations by HPLC, J. Pharm. Biomed. 29(4) (2002) 701- 714.
191. A. M. Di Piertra, R. Gatti, V. Andrisano, V. Cavrini, Application of HPLC with diode-array detection and on-line post column photochemical derivatization, J. Chromatography A 729(1-2) (1996) 355-361.
192. Susumu Yamato, Masaharu Nakajima, Kenji Shimada, Determination of chlorpheniramine by HPLC, J. Chromatography A 731(1-2) (1996) 346-350.
193. Nevin Erk, Murat Kartal, Determination of chlorpheniramine by HPLC and derivative ratio-spectra spectrophotometry, ll Farmaco, 53(8-9) (1998) 617-622.
194. Sabry Khalil, Determination of chlorpheniramine by atomic emission spectrophotometry, J. Pharm. Biomed. 21 (4) (1999) 697-702.
195. Nafisur Rahman, Sayed Nujmul Hejaz, Spectrophotometric determination of verapamil, ll Farmaco, 59(7) (2004) 529-536.

196. Yalçin Özkan, Niazi Yilmaz, Sibel A Özkan, Înci Biryol, Analysis of verapamil by HPLC and its application to dosage forms, ll Farmaco, 55(5) (2000) 376-382.
197. Sabry Khalil, Ahmed Kelzieh, Determination of verapamil by atomic emission spectrophotometry, J. Pharm. Biomed. 27 (1-2) (2002) 123-131.
198. Ensaf Aboul Kasim, M. A. Ghandour, M. T. El-Hatty, Mahasen M. Ahmed, Use of Adsorptive Stripping Voltametry for the determination of verapamil, J. Pharm. Biomed. 30 (4) (2002) 921-929.
199. J. A. Ortuño, C. Sánchez-Pedreño, A. Gil, Flow-injection Pulse Amperometric detection for the determination of verapamil, J. Anal. Chim. Acta. 554(1-2) (2005) 172-176.
200. M. Gil-Agusti, S. Carda-Broch, M. Elisa Capella-Peiro, Josep steve-Romero, Determination of five antianginals by Micellar Liquid chromatography, J. Pharm. Biomed. 41 (4) (2006) 1235-1242.
201. Joaquín A. Ortuño, Jorge Hernández, Concepción Sánchez-Pedreño, The use of ion-selective electrode for determination of multidrug resistance reversers, J. Sensors and Actuators B: Chemical 119 (1) (2006) 282-287.
202. Dongpo Xu, Shaopu Liu, Zhongfang Liu, Xiaoli Hu, Use of Resonance Rayleigh Scattering coupled with flow injection for the determination of verapamil, J. Anal. Chim. Acta. 588(1) (2007) 10-15.
203. Abu baker M. Idris, Fahad N. Assubaie Salah M. Sultan, Experimental design optimization of sequential injection method of promazine assay in bulk and pharmaceutical formulations, J. Auto. Methods and management in chemistry (2007) 1-7.
204.

Appendices

1. SIA program for washing all channels

'Flushing syringe

SyringePump Flowrate (microliter/sec) 150
Valve port 1
SyringePump Valve Out
SyringePump Empty
SyringePump Delay Until Done
'Washing the main channel
SyringePump Valve In
SyringePump Fill
SyringePump Delay Until Done
SyringePump Valve Out
SyringePump Empty
SyringePump Delay Until Done

'Washing tube connected with port 2
Valve port 2
SyringePump Aspirate (microliter) 1500
Valve port 1
SyringePump Empty
SyringePump Delay Until Done

'Washing tube connected with port 3
Valve port 3
SyringePump Aspirate (microliter) 1500
Valve port 1
SyringePump Empty
SyringePump Delay Until Done

'Washing tube connected with port 4
Valve port 4
SyringePump Aspirate (microliter) 1500
Valve port 1

SyringePump Empty

SyringePump Delay Until Done

'Washing tube connected with port 5

Valve port 5

SyringePump Aspirate (microliter) 1500

Valve port 1

SyringePump Empty

SyringePump Delay Until Done

'Washing tube connected with port 6

Valve port 6

SyringePump Aspirate (microliter) 1500

Valve port 1

SyringePump Empty

SyringePump Delay Until Done

'Washing tube connected with port 7

Valve port 7

Syringe Pump Aspirate (microliter) 1500

Valve port 1

Syringe Pump Empty

Syringe Pump Delay Until Done

'Washing tube connected with port 8

Valve port 8

Syringe Pump Aspirate (microliter) 1500

Valve port 1

Syringe Pump Empty

Syringe Pump Delay Until Done

2. SIA program for washing port 2

'Washing port 2

SyringePump Flowrate (microliter/sec) 150

SyringePump Valve Out
SyringePump Empty
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

'Sulphuric acid

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve Out
Valve port 2
SyringePump Aspirate (microliter) 100
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150
Valve port 1
SyringePump Empty
SyringePump Delay Until Done

3. SIA program for washing port 3

'Washing port 3

SyringePump Flowrate (microliter/sec) 150

SyringePump Valve Out
SyringePump Empty
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

'Permanganate

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve Out
Valve port 3
SyringePump Aspirate (microliter) 100
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150
Valve port 1
SyringePump Empty
SyringePump Delay Until Done

4. SIA program for washing port 4

'Washing port 4

SyringePump Flowrate (microliter/sec) 150

SyringePump Valve Out
SyringePump Empty
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

'Analyte

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve Out
Valve port 4
SyringePump Aspirate (microliter) 100
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150
Valve port 1
SyringePump Empty
SyringePump Delay Until Done

5. SIA program for washing and loading the channels

'Washing the main channel

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve Out

SyringePump Empty
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Fill
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150
Valve port 1
SyringePump Valve Out
SyringePump Empty
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

'Loading channels
'Sulfuric acid
SyringePump Flowrate (microliter/sec) 100
SyringePump Valve Out
Valve port 2
SyringePump Aspirate (microliter) 50
SyringePump Delay Until Done

'Permanganate
SyringePump Flowrate (microliter/sec) 100
Valve port 3
SyringePump Aspirate (microliter) 50

SyringePump Delay Until Done

'Chlorpheniramine

SyringePump Flowrate (microliter/sec) 100

Valve port 4

SyringePump Aspirate (microliter) 50

SyringePump Delay Until Done

'Spacer and Blank solution

SyringePump Flowrate (microliter/sec) 100

Valve port 5

SyringePump Aspirate (microliter) 50

SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150

Valve port 1

SyringePump Empty

SyringePump Delay Until Done

6. SIA program for controlling Verapamil

'Washing the main channel

SyringePump Flowrate (microliter/sec) 150

SyringePump Valve Out

SyringePump Empty
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Fill
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150
Valve port 1
SyringePump Valve Out
SyringePump Empty
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

'Loading channels
'Sulfuric acid
SyringePump Flowrate (microliter/sec) 50
SyringePump Valve Out
Valve port 2
SyringePump Aspirate (microliter) 50
SyringePump Delay Until Done

'Permanganate
SyringePump Flowrate (microliter/sec) 50
Valve port 3
SyringePump Aspirate (microliter) 50

SyringePump Delay Until Done

'Chlorpheniramine

SyringePump Flowrate (microliter/sec) 50

Valve port 4

SyringePump Aspirate (microliter) 50

SyringePump Delay Until Done

'Spacer and Blank solution

SyringePump Flowrate (microliter/sec) 50

Valve port 5

SyringePump Aspirate (microliter) 50

SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150

Valve port 1

SyringePump Empty

SyringePump Delay Until Done

'Blank 1

SyringePump Flowrate (microliter/sec) 150

SyringePump Valve In

SyringePump Aspirate (microliter) 1500

SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50

SyringePump Valve Out

Valve port 2

SyringePump Aspirate (microliter) 35

SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 3
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 5
SyringePump Aspirate (microliter) 50
SyringePump Delay Until Done
'Mixing reagents
SyringePump Flowrate (microliter/sec) 25
Valve port 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1

SyringePump Flowrate (microliter/sec) 50
Valve port 1
SyringePump Dispense (microliter) 250
SyringePump Delay Until Done

'Resident time

Delay (sec) 120

SyringePump Flowrate (microliter/sec) 25

SyringePump Dispense (microliter) 1200

Delay (sec) 1

Spectrometer Reference Scan

Delay (sec) 1

Spectrometer Absorbance Scanning

SyringePump Delay Until Done

Spectrometer Stop Scanning

SyringePump Flowrate (microliter/sec) 150

SyringePump Empty

SyringePump Delay Until Done

'Blank 2

SyringePump Flowrate (microliter/sec) 150

SyringePump Valve In

SyringePump Aspirate (microliter) 1500

SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50

SyringePump Valve Out

Valve port 2

SyringePump Aspirate (microliter) 35

SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50

Valve port 3

SyringePump Aspirate (microliter) 30

SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 5
SyringePump Aspirate (microliter) 50
SyringePump Delay Until Done

'Mixing reagents
SyringePump Flowrate (microliter/sec) 25
Valve port 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1

SyringePump Flowrate (microliter/sec) 50
Valve port 1
SyringePump Dispense (microliter) 250
SyringePump Delay Until Done

'Resident time
Delay (sec) 120

SyringePump Flowrate (microliter/sec) 25

SyringePump Dispense (microliter) 1200
Delay (sec) 1
Spectrometer Reference Scan
Delay (sec) 1
Spectrometer Absorbance Scanning
SyringePump Delay Until Done
Spectrometer Stop Scanning

SyringePump Flowrate (microliter/sec) 150
SyringePump Empty
SyringePump Delay Until Done

'Blank 3

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
SyringePump Valve Out
Valve port 2
SyringePump Aspirate (microliter) 35
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 3
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 5
SyringePump Aspirate (microliter) 50
SyringePump Delay Until Done

'Mixing reagents
SyringePump Flowrate (microliter/sec) 25
Valve port 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1

SyringePump Flowrate (microliter/sec) 50
Valve port 1
SyringePump Dispense (microliter) 250
SyringePump Delay Until Done

'Resident time
Delay (sec) 120

SyringePump Flowrate (microliter/sec) 25

SyringePump Dispense (microliter) 1200
Delay (sec) 1
Spectrometer Reference Scan
Delay (sec) 1
Spectrometer Absorbance Scanning
SyringePump Delay Until Done
Spectrometer Stop Scanning

SyringePump Flowrate (microliter/sec) 150
SyringePump Empty
SyringePump Delay Until Done
'Sample Meas. 1
SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
SyringePump Valve Out
Valve port 2
SyringePump Aspirate (microliter) 35
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 3
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done
SyringePump Flowrate (microliter/sec) 50
Valve port 4
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50

Valve port 5

SyringePump Aspirate (microliter) 20

SyringePump Delay Until Done

'Mixing reagents

SyringePump Flowrate (microliter/sec) 25

Valve port 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

'Pushing reagents to the reaction coil

SyringePump Flowrate (microliter/sec) 50

Valve port 1

SyringePump Dispense (microliter) 250

SyringePump Delay Until Done

Delay (sec) 5

'Resident time

Delay (sec) 120

SyringePump Flowrate (microliter/sec) 25

SyringePump Dispense (microliter) 1200
Delay (sec) 1
Spectrometer Reference Scan
Delay (sec) 1
Spectrometer Absorbance Scanning
SyringePump Delay Until Done
Spectrometer Stop Scanning

SyringePump Flowrate (microliter/sec) 150
SyringePump Empty
SyringePump Delay Until Done
'Sample Meas. 2
SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
SyringePump Valve Out
Valve port 2
SyringePump Aspirate (microliter) 35
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 3
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done
SyringePump Flowrate (microliter/sec) 50
Valve port 4
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50

Valve port 5

SyringePump Aspirate (microliter) 20

SyringePump Delay Until Done

'Mixing reagents

SyringePump Flowrate (microliter/sec) 25

Valve port 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

'Pushing reagents to the reaction coil

SyringePump Flowrate (microliter/sec) 50

Valve port 1

SyringePump Dispense (microliter) 250

SyringePump Delay Until Done

Delay (sec) 5

'Resident time

Delay (sec) 120

SyringePump Flowrate (microliter/sec) 25

SyringePump Dispense (microliter) 1200
Delay (sec) 1
Spectrometer Reference Scan
Delay (sec) 1
Spectrometer Absorbance Scanning
SyringePump Delay Until Done
Spectrometer Stop Scanning

SyringePump Flowrate (microliter/sec) 150
SyringePump Empty
SyringePump Delay Until Done
'Sample Meas. 3
SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
SyringePump Valve Out
Valve port 2
SyringePump Aspirate (microliter) 35
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 3
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done
SyringePump Flowrate (microliter/sec) 50
Valve port 4
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50

Valve port 5

SyringePump Aspirate (microliter) 20

SyringePump Delay Until Done

'Mixing reagents

SyringePump Flowrate (microliter/sec) 25

Valve port 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

'Pushing reagents to the reaction coil

SyringePump Flowrate (microliter/sec) 50

Valve port 1

SyringePump Dispense (microliter) 250

SyringePump Delay Until Done

Delay (sec) 5

'Resident time

Delay (sec) 120

SyringePump Flowrate (microliter/sec) 25

SyringePump Dispense (microliter) 1200

Delay (sec) 1

Spectrometer Reference Scan

Delay (sec) 1

Spectrometer Absorbance Scanning

SyringePump Delay Until Done

Spectrometer Stop Scanning

SyringePump Flowrate (microliter/sec) 150

SyringePump Empty

SyringePump Delay Until Done

7. SIA program for controlling Chlorpheniramine

'Washing the main channel

SyringePump Flowrate (microliter/sec) 150

Valve port 1

SyringePump Valve Out

SyringePump Empty

SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec)150

SyringePump Valve In

SyringePump Aspirate (microliter) 1500

SyringePump Delay Until Done

'Loading channels

'Sulfuric acid

SyringePump Flowrate (microliter/sec) 50

SyringePump Valve Out

Valve port 2

SyringePump Aspirate (microliter) 50

SyringePump Delay Until Done

'Permanganate

SyringePump Flowrate (microliter/sec) 50

Valve port 3

SyringePump Aspirate (microliter) 50

SyringePump Delay Until Done

'Chlorpheniramine

SyringePump Flowrate (microliter/sec) 50

Valve port 4

SyringePump Aspirate (microliter) 50

SyringePump Delay Until Done

'Spacer and Blank solution

SyringePump Flowrate (microliter/sec) 50

Valve port 5

SyringePump Aspirate (microliter) 50

SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 150
Valve port 1
SyringePump Empty
SyringePump Delay Until Done

'Blank 1

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
SyringePump Valve Out
Valve port 2
SyringePump Aspirate (microliter) 35
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 3
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 5
SyringePump Aspirate (microliter) 50
SyringePump Delay Until Done

'Mixing reagents

SyringePump Flowrate (microliter/sec) 25
Valve port 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1

SyringePump Flowrate (microliter/sec) 50
Valve port 1
SyringePump Dispense (microliter) 250
SyringePump Delay Until Done

'Resident time
Delay (sec) 240

SyringePump Flowrate (microliter/sec) 25
SyringePump Dispense (microliter) 1200
Delay (sec) 1
Spectrometer Reference Scan

Delay (sec) 1
Spectrometer Absorbance Scanning
SyringePump Delay Until Done
Spectrometer Stop Scanning

SyringePump Flowrate (microliter/sec) 150
SyringePump Empty
SyringePump Delay Until Done

'Blank 2

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
SyringePump Valve Out
Valve port 2
SyringePump Aspirate (microliter) 35
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 3
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 5
SyringePump Aspirate (microliter) 50
SyringePump Delay Until Done

'Mixing reagents

SyringePump Flowrate (microliter/sec) 25

Valve port 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Flowrate (microliter/sec) 50

Valve port 1

SyringePump Dispense (microliter) 250

SyringePump Delay Until Done

'Resident time

Delay (sec) 240

SyringePump Flowrate (microliter/sec) 25

SyringePump Dispense (microliter) 1200

Delay (sec) 1

Spectrometer Reference Scan

Delay (sec) 1
Spectrometer Absorbance Scanning
SyringePump Delay Until Done
Spectrometer Stop Scanning

SyringePump Flowrate (microliter/sec) 150
SyringePump Empty
SyringePump Delay Until Done

'Blank 3

SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
SyringePump Valve Out
Valve port 2
SyringePump Aspirate (microliter) 35
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 3
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 5
SyringePump Aspirate (microliter) 50
SyringePump Delay Until Done

'Mixing reagents

SyringePump Flowrate (microliter/sec) 25
Valve port 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1
SyringePump Dispense (microliter) 10
Delay (sec) 1
SyringePump Aspirate (microliter) 10
Delay (sec) 1

SyringePump Flowrate (microliter/sec) 50
Valve port 1
SyringePump Dispense (microliter) 250
SyringePump Delay Until Done

'Resident time
Delay (sec) 240

SyringePump Flowrate (microliter/sec) 25
SyringePump Dispense (microliter) 1200
Delay (sec) 1
Spectrometer Reference Scan

Delay (sec) 1
Spectrometer Absorbance Scanning
SyringePump Delay Until Done
Spectrometer Stop Scanning

SyringePump Flowrate (microliter/sec) 150
SyringePump Empty
SyringePump Delay Until Done

'Sample Meas. 1
SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
SyringePump Valve Out
Valve port 2
SyringePump Aspirate (microliter) 35
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 3
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 4
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50

Valve port 5

SyringePump Aspirate (microliter) 20

SyringePump Delay Until Done

'Mixing reagents

SyringePump Flowrate (microliter/sec) 25

Valve port 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

'Pushing reagents to the reaction coil

SyringePump Flowrate (microliter/sec) 50

Valve port 1

SyringePump Dispense (microliter) 250

SyringePump Delay Until Done

'Resident time

Delay (sec) 240

SyringePump Flowrate (microliter/sec) 25

SyringePump Dispense (microliter) 1200
Delay (sec) 1
Spectrometer Reference Scan
Delay (sec) 1
Spectrometer Absorbance Scanning
SyringePump Delay Until Done
Spectrometer Stop Scanning

SyringePump Flowrate (microliter/sec) 150
SyringePump Empty
SyringePump Delay Until Done
'Sample Meas. 2
SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
SyringePump Valve Out
Valve port 2
SyringePump Aspirate (microliter) 35
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 3
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done
SyringePump Flowrate (microliter/sec) 50
Valve port 4
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50

Valve port 5

SyringePump Aspirate (microliter) 20

SyringePump Delay Until Done

'Mixing reagents

SyringePump Flowrate (microliter/sec) 25

Valve port 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

'Pushing reagents to the reaction coil

SyringePump Flowrate (microliter/sec) 50

Valve port 1

SyringePump Dispense (microliter) 250

SyringePump Delay Until Done

'Resident time

Delay (sec) 240

SyringePump Flowrate (microliter/sec) 25

SyringePump Dispense (microliter) 1200
Delay (sec) 1
Spectrometer Reference Scan
Delay (sec) 1
Spectrometer Absorbance Scanning
SyringePump Delay Until Done
Spectrometer Stop Scanning

SyringePump Flowrate (microliter/sec) 150
SyringePump Empty
SyringePump Delay Until Done
'Sample Meas. 3
SyringePump Flowrate (microliter/sec) 150
SyringePump Valve In
SyringePump Aspirate (microliter) 1500
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
SyringePump Valve Out
Valve port 2
SyringePump Aspirate (microliter) 35
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50
Valve port 3
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done
SyringePump Flowrate (microliter/sec) 50
Valve port 4
SyringePump Aspirate (microliter) 30
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50

Valve port 5

SyringePump Aspirate (microliter) 20

SyringePump Delay Until Done

'Mixing reagents

SyringePump Flowrate (microliter/sec) 25

Valve port 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

SyringePump Dispense (microliter) 10

Delay (sec) 1

SyringePump Aspirate (microliter) 10

Delay (sec) 1

'Pushing reagents to the reaction coil

SyringePump Flowrate (microliter/sec) 50

Valve port 1

SyringePump Dispense (microliter) 250

SyringePump Delay Until Done

'Resident time

Delay (sec) 240

SyringePump Flowrate (microliter/sec) 25

SyringePump Dispense (microliter) 1200

Delay (sec) 1

Spectrometer Reference Scan

Delay (sec) 1

Spectrometer Absorbance Scanning

SyringePump Delay Until Done

Spectrometer Stop Scanning

SyringePump Flowrate (microliter/sec) 150

SyringePump Empty

SyringePump Delay Until Done

8. SIA program to expel the bubbles

'Washing the main channel

SyringePump Flowrate (microliter/sec) 150

Valve port 1

SyringePump Valve Out
SyringePump Empty
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 200
SyringePump Valve In
SyringePump Fill
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 200
Valve port 1
SyringePump Valve Out
SyringePump Empty
SyringePump Delay Until Done